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CONTROL OF SNOW MOLDING IN CONIFEROUS NURSERY STOCK [1]

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During the time that the Cottonwood Nursery of the United States Forest Service was in operation (1906–1921) very serious winter losses of Douglas fir (*Pseudotsuga taxifolia*) seedlings and transplants occurred under the snow. Several other species were also injured, the extent of loss varying with the species, age class, and time of snow disappearance in the spring. All age classes of Norway spruce (*Picea excelsa*) were especially susceptible, while Engelmann spruce (*Picea engelmanni*) and the Pacific coast form of western yellow pine (*Pinus ponderosa*) suffered only moderate injury, and lodgepole pine (*Pinus contorta*) was practically immune.

The Cottonwood Nursery is situated 25 miles southeast of Salt Lake City, Utah, on the Wasatch National Forest, at an elevation of 7,450 feet. Here the snow usually covers the ground continuously from November 1 to May 10, lasting occasionally even until May 20 or 25. The maximum depth varies from 6 to 8 feet. Under this heavy blanket of snow the ground seldom freezes, and even where freezing has occurred in the autumn before the advent of permanent snow, the soil soon thaws out after the snow begins to accumulate. Invariably the injury was greatest during years of heaviest snowfall and consequent late melting of snow in the spring.

Hartley, Pierce, and Hahn [3] studied this type of injury and found that it was caused by weakly parasitic fungi, attacking the leaves. They succeeded in isolating, by cultural methods, a number of organisms from recently snow-molded seedlings of Douglas fir, of which they most strongly suspected *Botrytis cinerea* and a dark sterile mold, as yet unidentified, as causing the disease. These investigators conducted spraying experiments with fungicides, including sulphuric acid, formaldehyde, zinc chloride, copper sulphate, copper acetate, and ammoniacal copper carbonate, none of which gave promise of effectively controlling the disease. A lime-sulphur mixture gave only very slightly beneficial results. Mulches consisting of thin layers of sawdust, sterilized sand, and gravel gave no promise. At the beginning of the present investigation

[1] Accepted for publication Sept. 2, 1922.
[2] The writer is indebted to former Forest Planting Assistant N. J. Fetherolf for painstaking assistance in carrying out the experiments here reported.
[3] HARTLEY, Carl, PIERCE, Roy G., and HAHN, Glenn G. MOULDING OF SNOW-SMOTHERED NURSERY STOCK. *In* Phytopathology, v. 9, p. 521–531. 1919.

the writer submitted diseased specimens to Dr. Carl Hartley, who reported that the organisms were evidently the same as those with which he had worked.

For several years the practice of sowing black soil on the snow early in the spring to hasten its melting was followed by the nurserymen, but even with this practice the losses were still so heavy that the growing of Douglas fir on a large scale at the Cottonwood Nursery was discontinued in 1916.

TABLE I.—*Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1920*

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	*Per cent.*
Picea engelmanni	1–0	31.3	0
	2–0	27.7	0
	2–1	9.9	0
	3–2	8.1	0
Picea excelsa	2–0	77.0	0
	2–1	63.0	22.3
	3–2	59.3	0
Pseudotsuga taxifolia	1–0	62.9	0
	2–0	62.7	0
Pinus ponderosa	1–0	93.2	6.8
	2–0	52.2	44.7
	2–1	15.2	12.3
Pinus ponderosa scopulorum	1–0	16.8	2.5
	2–0	7.8	3.4
	2–1	3.5	15.8

The extent of the injury by these fungi among the different species and age classes is shown in Tables I and II, which are based upon counts of from several hundred to as many as 2,400 seedlings in each age class. It is recognized that a small loss occurred during the summer seasons but it was found to be negligible in comparison with the loss due to snow molding. Table I and Plate 1 show the much greater susceptibility of the exotic Norway spruce to the disease as compared with that of the native Engelmann spruce. Table I shows quite clearly that the younger age classes, 1–0 and 2–0, suffer by far the greatest loss and injury. Table II fails to bring out this relationship so clearly mainly because of the fact that a large part of the older age classes of stock had been abandoned, receiving no water or care of any kind during the summer of 1920. This left the stock in a weakened condition and an easy prey for the fungi. For example, 3–3 Engelmann spruce shows (not in Table II) 41.5 per cent of dead and injured trees as compared with 3.4 per cent for the 3–2 stock and 7.1 per cent for the 3–1 stock. The first age class had been abandoned in 1920, while the latter two classes of stock were watered and otherwise properly cared for during 1920. It should be noted, therefore, that among the older age classes, especially transplants, it is usually the weak or previously injured trees that are killed or severely injured. It is not uncommon to find some of the leaves of vigorous transplants infected when the snow melts, but the development of the fungi is checked as soon as the trees are exposed to the sunlight, and complete recovery may follow. Jack pine (*Pinus banksiana*), however,

was found to be a notable exception. During the winter of 1919–1920 a bed of some 300 vigorous 4-year-old jack pine transplants, 18 inches tall, was completely killed by the disease. Tables I and II clearly show that the Pacific Coast form of western yellow pine (*Pinus ponderosa*), grown from seed collected in central Idaho, is much more susceptible to the snow-molding fungi than the Rocky Mountain variety of the same species (*Pinus ponderosa scopulorum*), grown from seed collected in the region near the nursery.

TABLE II.—*Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1921*

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	*Per cent.*
Picea engelmanni	3–0	8. 4	13. 3
	3–1	0. 8	6. 3
	3–2	0	3. 4
Picea excelsa	3–0	80. 4	11. 7
	3–3	80. 3	19. 6
Picea parryana	3–0	60. 2	30. 8
	3–3	3. 0	31. 5
Pseudotsuga taxifolia	1–0	5. 1	6. 9
	2–0	16. 2	19. 3
	[a] 3–0	2. 0	5. 4
	[b] 3–0	31. 1	30. 4
Pinus ponderosa	1–0	11. 5	3. 5
	3–0	0	83. 0
	2–1	37. 2	38. 0
	2–2	36. 8	35. 6
Pinus ponderosa scopulorum	1–0	0. 8	1. 6
	3–0	4. 5	18. 5
	2–1	23. 7	26. 8
	2–2	6. 3	24. 7
Pinus contorta	2–1	0	3. 0
	2–2	0	1. 5
Abies concolor	3–0	22. 1	30. 8
Pinus resinosa	2–1	0	15. 8
Pinus strobus	2–1	60. 2	39. 8
Libocedrus decurrens	.....	4. 1	8. 4
Thuja occidentalis	.....	100. 0	0
Juniperus monosperma	.....	100. 0	0

[a] Bed protected by framework of 2 by 6 lumber. [b] Unprotected bed.

Since most fungi will gain a foothold more readily at some previously injured part of a plant, it seems quite probable that the fungous injury during the winter of 1919–1920 was greatly increased as a result of the severe injury to the ends of the twigs by frost in the spring of 1919.[4]

In the spring of 1918 the problem of working out an efficacious method of controlling the snow molding of coniferous nursery stock was taken up by the writer. Six 4 by 12 foot beds were sown to the same amounts of Douglas fir, the time and method of sowing and subsequent care throughout the summer season being the same. Uniformly good stands were secured on all of the beds. The seed beds were covered with shade frames giving half shade during the hot, dry portion of the summer. In the autumn the shade frames were removed and just before the

[4] KORSTIAN, Clarence F. EFFECT OF A LATE SPRING FROST UPON FOREST VEGETATION IN THE WASATCH MOUNTAINS OF UTAH. *In* Ecology, v. 2, p. 47–52, 1 fig. 1921. Literature cited, p. 52.

permanent winter snow came, different treatments were applied to five of the beds, while the sixth was left untreated as a control. The object of these special treatments was mainly to afford mechanical protection from the disease. The treatment which gave much the best results consisted of a framework of 2 by 6 inch planks loosely placed with a spacing of 1 to 2 inches upon logs or squared timbers lying lengthwise along the sides of the beds. This type of protective covering, which was used on Bed 14, is shown in Plate 2, B. Two-inch lumber was used, mainly because it was available. It is possible that one-inch lumber could be used, if it were adequately supported to hold the heavy mass of snow above the tops of the seedlings. As soon as the snow had melted sufficiently in the spring to permit, the framework was removed. It was placed on the bed each autumn before the advent of the heavy winter snowfall.

TABLE III.—*Survival of Douglas fir seedlings in beds receiving different treatments as indicated; sowing spring of 1918.*

Bed No.	Date of count.	Total germination.	Survival.		Special treatment.
			Total.	In per cent of total germination.	
		Per square foot.	*Per square foot.*	*Per cent.*	
12	Oct. 9, 1918	126	103	81.8	None; control.
	Sept. 29, 1919		81	64.2	
	July 1, 1920		45	35.7	
	Oct. 6, 1920		42	33.3	
	May 22, 1921		14	11.1	
13	Oct. 9, 1918	163	131	80.3	Mulched with timothy hay winters of 1918–19 and 1919–20, 1920–21.
	Sept. 29, 1919		24	14.7	
	July 1, 1920		0	0	
	Oct. 6, 1920		0	0	
	May 22, 1921		0	0	
14	Oct. 9, 1918	128	98	76.6	Framework of 2 by 6's, 2 inches apart supported by timbers several inches above tops of trees. Winters 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		95	74.2	
	July 1, 1920		84	60.0	
	Oct. 6, 1920		84	60.0	
	May 22, 1921		84	60.0	
15	Oct. 9, 1918	69	58	84.0	Mulched with aspen leaves. Winters 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		24	34.8	
	July 1, 1920		13	18.8	
	Oct. 6, 1920		13	18.8	
	May 22, 1921		13	18.8	
16	Oct. 9, 1918	79	58	73.4	Covered with canvas throughout the winters of 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		28	35.4	
	July 1, 1920		3	3.8	
	Oct. 6, 1920		3	3.8	
	May 22, 1921		1	1.3	
17	Oct. 9, 1918	134	96	71.6	Mulched with sterilized sand. Winters of 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		78	58.2	
	July 1, 1920		8	6.0	
	Oct. 6, 1920		8	6.0	
	May 22, 1921		5	3.7	
7	Oct. 6, 1920	165	[a] 110	66.6	Framework, 2 by 6's.
	May 22, 1921		[a] 100	60.6	Same as bed No. 14.
9	Oct. 6, 1920	142	[a] 93	65.4	None; control.
	May 22, 1921		[a] 23	16.2	

[a] Sowing spring of 1920.

Since this investigation was completed Cornefert [5] has reported a somewhat different method which has given excellent results for several years in the protection of spruce nursery stock from *Herpotrichia nigra*. In the autumn peeled poles 10 to 15 cm. in diameter are placed between the rows of plants, so that the plants lean upon them when pressed down by the snow. In field planting each tree is placed so that it can be supported by a rock or a stump.

Table III shows the original germination per square foot, survival on various dates, and the special treatment applied to each of the beds sown in 1918. The use of the protective framework of 2 by 6 inch planks was again checked during the winter of 1920–21 on Douglas fir seedlings originating from seed sown in the spring of 1920, the survival data for which is also included in Table III. The general appearance of the 3-0 beds of the 1918 sowing are shown in Plate 2, A. From Table III it will be seen that the results are decidedly in favor of the plank framework (Bed 14 of the 1918 sowing and Bed 7 of the 1920 sowing), which keeps the heavy mass of snow from mashing the seedlings down flat against the soil. Sufficient snow sifts through the intervening spaces between the 2 by 6's to keep the soil moist during the winter. At the end of the third winter Bed 14 of the 1918 sowing showed an average survival of 84 seedlings per square foot, or 60 per cent of the original germination compared with the next highest survival of 14 seedlings per square foot, or 11.1 per cent of the original germination in Bed 12—the control bed to which no special treatment had been applied. During the winters of 1918–19 and 1919–20 the protected bed (No. 14) suffered losses of 2.4 per cent and 14.2 per cent and none at all during the succeeding winter, as compared with losses in the control bed (No. 12) during the three winters of 17.6, 28.5, and 22.2 per cent, respectively. Bed 7 of the 1920 sowing, which was given protection by the same type of framework covering as that described above, suffered a loss of 6 per cent during the winter of 1920–21, as compared with a loss of 49.2 per cent in the control bed during the same winter.

It is evident from Table III and from general observations in the nursery that a mulch of organic matter stimulates the activities of the fungi by providing a suitable substratum on which they may develop. Contact with various kinds of organic matter, dead snow-molded plants, and a surface even with the soil predisposes to the disease.

On May 25, 1921, the stock in Bed 14 (protected by framework) and in Bed 12 (control) was dug and graded with the result that 67 per cent of the stock from Bed 14 was suitable for transplanting, while only 34.4 per cent of the seedlings from Bed 12 was suitable. Even many of the firsts in the unprotected bed were infected with the snow-molding fungi, which probably would result in a heavier culling the following spring when the transplants were dug preparatory to field planting. The foliage was of a dull green color and numerous leaves were dead or partially killed. This lot of stock had very few healthy buds, only 25.5 per cent, while the seedlings from the protected bed had 90.7 per cent of good terminal buds. In each case the bud scales of the unhealthy seedlings were loose and flaccid, giving the buds a withered appearance. Small bunches of each lot of stock were allowed to stand in the laboratory with the roots submerged in water for 10 days. Practically all of the buds on the seedlings

[5] CORNEFERT, R. RÉGÉNÉRATION SUR LES HAUTS PLATEAUX DU JURA. *In* Soc. Forest. Franche-Comté et Belfort Bul. Trimest., t. 14, p. 206. 1921.

from the protected bed developed into normal growing shoots, while those from the unprotected bed remained in a dormant condition, apparently due to injury by these fungi.

Table IV and Plate 3 show the root, top, and terminal bud development of Douglas fir seedlings grown three years in protected and unprotected seed beds. The trees from the unprotected bed had heavier roots and tops and a greater stem diameter. This is probably due to the fact that the stand of seedlings in the unprotected bed is much more open than in the protected bed, and consequently each plant has more growing space in which to develop. At the same time none but the largest and most vigorous seedlings survived in the unprotected bed. On the other hand, the roots from the unprotected bed were heavier in proportion to the weight of the plant, the tops being correspondingly less. This is probably attributable to the fungous injury to the tops, which was unfavorable to their development and which was much less pronounced in the protected beds.

TABLE IV.—*Root, top, and terminal bud development*

Bed.	Average length of tops.	Average length of roots.	Average weight of tops.	Average weight of roots.	Ratio of weight of root to weight of entire plant.	Average diameter of stem at root collar.	Average length of terminal bud.	Average width of terminal bud.
	Inches.	*Inches.*	*Gm.*	*Gm.*	*Per cent.*	*Inches.*	*Inches.*	*Inches.*
Protected seedlings (Bed 14).....	6.03	11.43	1.62	0.46	22.1	0.08	0.24	0.11
Unprotected seedlings (Bed 12).....	5.77	10.68	1.78	.75	29.6	.10	.18	.09

The examination of field plantations revealed considerable fungous injury during the winter of 1919–1920, especially among jack pine, Engelmann spruce and Norway spruce. The jack pine were practically all dead and the few living trees so severely injured that ultimate recovery was impossible. With Norway spruce and Engelmann spruce, the greatest injury from these fungi occurred on soils rich in organic matter, the surface of which was covered with considerable plant litter. The underplanting of aspen (*Populus tremuloides*) stands with Engelmann spruce, Norway spruce, and Douglas fir has been accompanied by notable losses on areas on which the ground was covered with a deep layer of aspen leaves. The heaviest losses occur where the plants are bent over into intimate contact with the soil and litter which is the breeding ground of these weakly parasitic snow-molding fungi. Serious loss by molding under the snow can be prevented in spring plantings by removing the accumulation of leaf litter from an area of one or two square feet, in the center of which the hole for the tree is dug and also by the use of large vigorous planting stock, which will stand erect during leaf fall and through the wet snows of the early winter and in this way hold its leader out of the zone of greatest danger.

CONCLUSIONS

The snow-molding fungi have caused a greater loss of coniferous nursery stock at the Cottonwood Nursery than any other form of injury or disease.

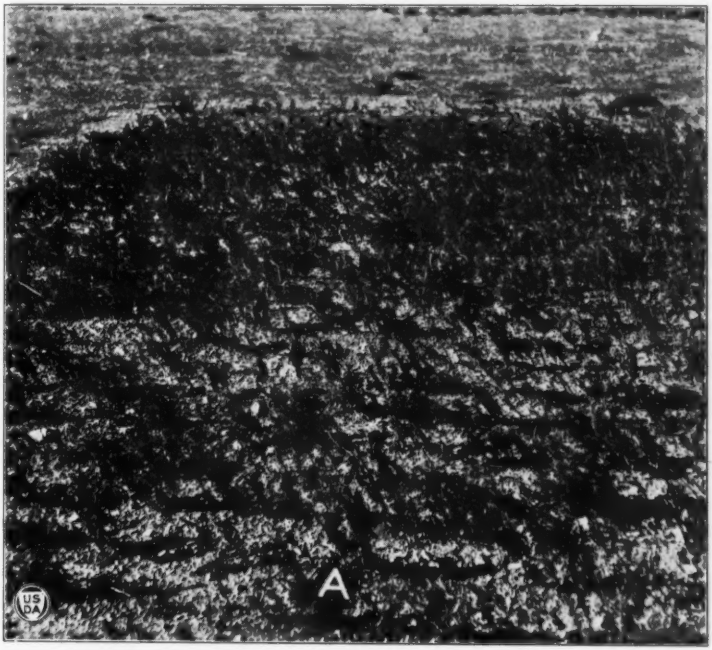
Examinations showed that practically all age classes and species grown in this nursery, excepting lodgepole pine, are subject to this disease and that it is more prevalent among young seedlings (1–0 and 2–0) than in the older age classes. Among older seedlings and transplants, the weak and previously injured trees are the ones generally attacked.

The disease can be controlled effectively in the nursery by placing a protective framework over the bed so that the full weight of the snow does not lie heavily on the trees, and press them down flat on the ground. It is by far the best and most efficient method of control which has been developed.

PLATE 1

A.—Bed of 3–1 Norway spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing the very high mortality and large amount of injury due to snow-molding fungi.

B.—Bed of Engelmann spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing its relative freedom from snow-molding injury, as compared with Norway spruce.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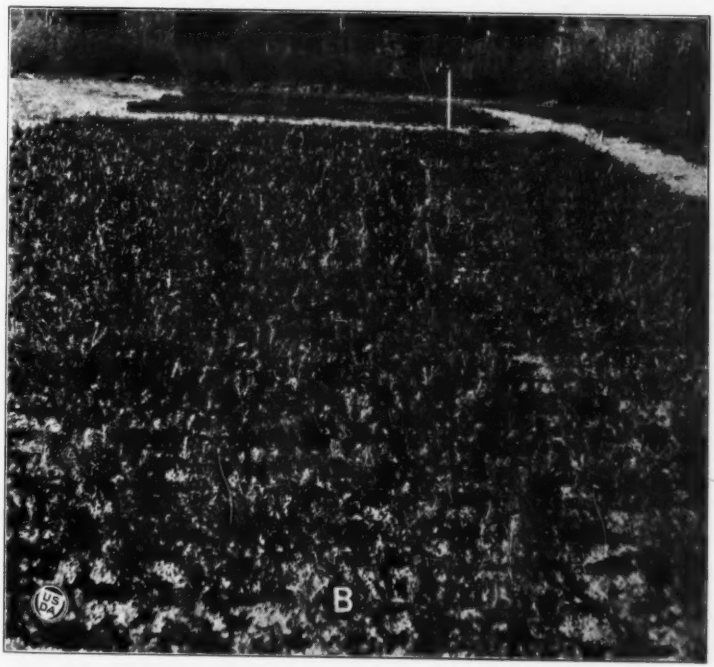
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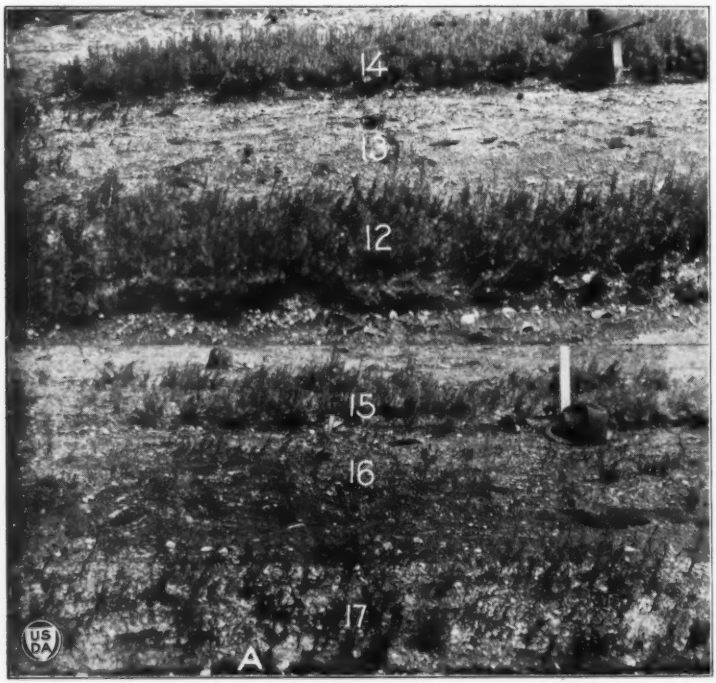
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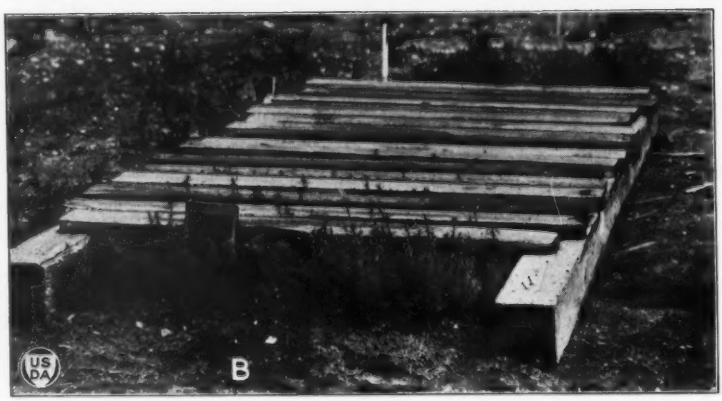
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PLATE 2

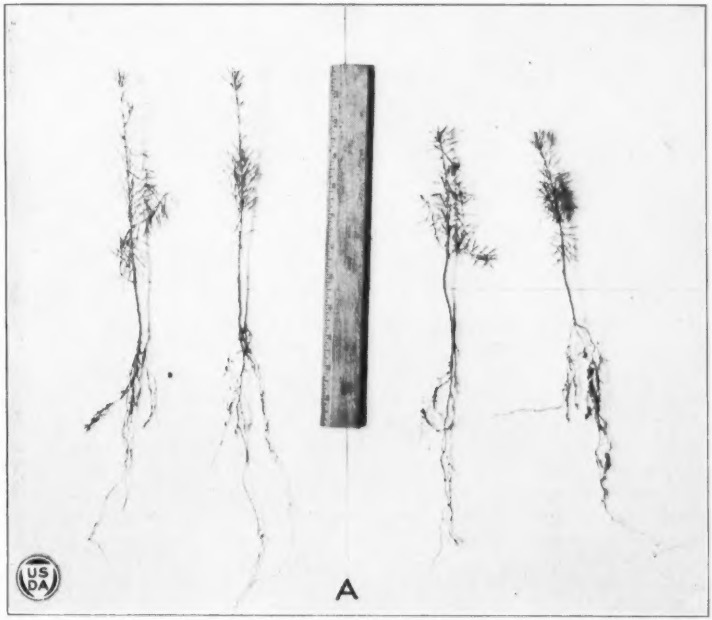
A.—Beds 12, 13, 14, 15, 16, and 17, 3–0 Douglas fir seedlings, as they appeared on May 27, 1921, about two weeks after the snow had disappeared. Bed 12 (control) received no special treatment, Bed 13 was mulched with timothy hay, Bed 14 was protected with a plank framework, Bed 15 was mulched with aspen leaves, Bed 16 was covered with canvas throughout the winter, Bed 17 was mulched with sterilized sand each autumn. Note the very poor stand on the mulched beds and the good stand and fine appearance of the protected bed (No. 14), as compared with the control bed (No. 12).

B.—The protective framework of 2 by 6's used on Bed 14, with a few of them removed from the front of the bed to show the 3–0 Douglas fir seedlings. Round logs may be used successfully instead of square timbers.

PLATE 3

A.—Healthy 3–0 Douglas fir seedlings from Bed 14, protected by framework of 2 by 6 planks, in which there was practically no injury from snow-molding fungi.

B.—Three-year-old Douglas fir seedlings from Bed 12, unprotected, showing unhealthy appearance due to injury from snow-molding fungi.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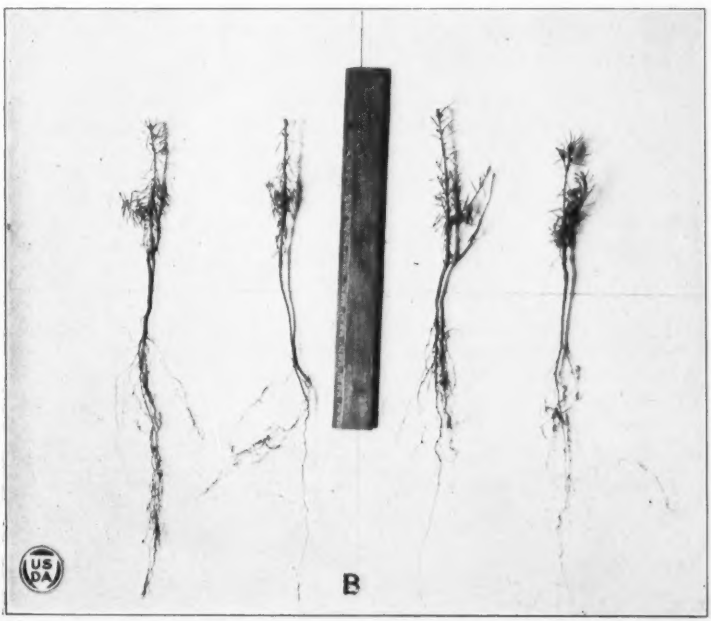
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AN INFLUENCE OF MOISTURE ON BEAN WILT[1]

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In 20 years' experience with the inoculation of legumes there have arisen only two noticeable failures in field work which could not be attributed to soil or climatic conditions or to the lack of efficiency on the part of the culture involved. The first case occurred with cowpea, evidenced by normal growth in the plants from untreated seed and sickly plants from the inoculated cowpeas. The other failure occurred in conjunction with the inoculation of navy bean, *Phaseolus vulgaris*, on the farm of the Office of Forage Crop Investigations at Redfield, S. Dak., in 1920 and was brought to my attention by Messrs. R. A. Oakley and H. L. Westover of that office. In the latter case many of the plants from beans which had been treated with a pure liquid culture, such as is distributed by the Department of Agriculture, died during the growing season, and the damage was sufficiently extensive to be rather noticeable in comparison with the rows of plants from the seed planted in the dry condition. It was estimated that 90 per cent of the crop was killed among the treated beans, whereas the loss was very slight among the plants which were uninoculated. A duplicate experiment with two different cultures, made on different pieces of ground at Redfield in 1921, gave an average loss of 25 per cent, due to treatment with liquid culture.

The possibility that the bean legume organism assumes a definite pathological rôle was considered, but this theory has been completely discarded since the publication of the work of Miss Florence Hedges,[3] who isolated and described an entirely different organism as the cause of this disease. The material used by Miss Hedges was obtained at Redfield, S. Dak.

The elimination of the possibility first mentioned led to the consideration of a second theory regarding the prevalence of the disease—that is, that some constituent of the medium employed to grow the culture of legume bacteria exercises a stimulating influence on the pathogenic organisms already in or on the seed. Miss Hedges found that the causative organism of the disease in question was generally present in the seeds which were used in the experiment.

EXPERIMENTAL PLAN

Attempts made to reproduce this diseased condition by the application of water and solutions containing the constituents of the media to beans which were planted on the Arlington Experimental Farm, Rossyln, Va., gave practically no definite results in one year, despite

[1] Accepted for publication Sept. 2, 1922.

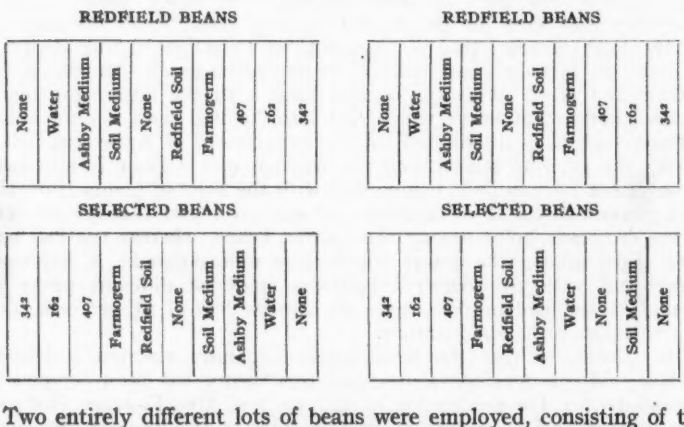
[2] A large amount of credit for the successful carrying out of this experiment is due to Mr. H. L. Westover and Mr. Samuel Garver of the Office of Forage Crop Investigations.

[3] HEDGES, Florence. A BACTERIAL WILT OF THE BEAN CAUSED BY BACTERIUM FLACCUMFACIENS NOV. SP. In Science, n. s., v. 55, p. 433-434. 1922.

the fact that the seed and treatment were similar to those employed at Redfield, S. Dak. Results similarly inconclusive were obtained in 1922 at Rossyln, Va., from an experiment planned to parallel the one at Redfield the same year. It became necessary, therefore, to collect in South Dakota, entirely, the data concerning this work.

The plan of experiment was as follows: Allowance was made for duplicate rows of each treatment with each lot of beans and quadruplicate rows of untreated beans; rows were 3 feet apart and approximately 66 feet long; observations were made on nodule formation and the presence of disease.

Plan of Navy Bean Experiment at Redfield, S. Dak., in 1922



Two entirely different lots of beans were employed, consisting of the unsorted Redfield seed, the progeny of seed which gave such disastrous results in previous experiments, and a lot purchased from a seedsman of Washington, D. C., which was sorted with special care to remove such beans as gave evidence of harboring the bean wilt organism. Yellow spots on the beans, indicative of the disease, were more prevalent in the Redfield seed.

The two cultures of *B. radicicola*, bean strain, one of which was used in 1920, and both of which were tried in 1921, were supplemented by a commercial culture and one from Wisconsin.[4] All cultures were liquid except the commercial culture, which was agar, but for application to seed it was necessary to add water.

Since it was not known which of two media was used for growing the cultures employed in the 1920 and 1921 experiments, it was necessary to include both Ashby[5] broth and soil broth made from a modified formula of Löhnis[6] in the experiment as sterile control media. The latter medium, however, was used for the propagation of the cultures of the bean nodule organism. Soil from Redfield which had produced bean plants with nodules on their roots under greenhouse conditions comprised the source of soil inoculation.

[4] Kindly supplied by Dr. E. B. Fred, University of Wisconsin, Madison, Wis.

[5] ASHBY, S. F. SOME OBSERVATIONS ON THE ASSIMILATION OF ATMOSPHERIC NITROGEN BY A FREE LIVING SOIL ORGANISM.—AZOTOBACTER CHROOCOCCUM OF BEIJERINCK. *In* Jour. Agr. Sci., v. 2, p. 38. 1907.

[6] LÖHNIS, F. LABORATORY METHODS IN AGRICULTURAL BACTERIOLOGY. Tr. by Wm. Stevenson and J. H. Smith. xi, 136 p., 40 fig., 3 pl. 1913.

APPLICATION OF MATERIALS

The seed was divided into portions suitable for sowing an individual row. To each lot of seed was added its respective preparation in quantity sufficient to allow for the complete moistening of the beans. After they were treated they were spread out to dry; following this operation, they were planted. All seed used in the experiment was planted by hand on May 26, 1922, care being taken to cover the seed promptly to prevent the undue exposure of the inoculated seed to the rays of the sun. The soil which was practically bone-dry, was sowed at the approximate rate of 1,000 pounds per acre in the rows with the seed and covered promptly.

BASES OF OBSERVATIONS

Wilting symptoms (Pl. 1, B) were taken as the criteria for collecting disease data. Hand sections of the stems of the bean plants in the wilting stage invariably showed the presence of numerous bacteria in the tissues, and even in the healthy appearing tissues of these plants bacteria were noticeable. The wilting condition occurred either in the main stem or side shoot or in both (Pls. 1, A, B, and 2, A, B) and at practically all periods of growth. In connection with the figures reported in Table I it should be mentioned that dead plants were assumed to have been killed by the wilt disease organism. In a few cases partial recovery was noted, but only for a very short time. These data were collected on July 24 and July 25.

TABLE I.—*Influence of moisture on the bacterial wilt of navy beans in Redfield, S. Dak., soils*

Treatment.	Redfield seed.			Selected market seed.		
	Number of plants.	Diseased.	Totally diseased.	Number of plants.	Diseased.	Totally diseased.
		Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
None [1]	933	2.0	0.7	1,042	2.9	0.1
Water	378	19.5	7.8	383	6.8	1.3
Ashby broth	365	22.0	10.3	475	5.6	.0
Lohnis broth	323	15.3	7.0	440	5.0	.6
Inoculated soil	481	7.0	3.4	507	2.8	.2
Commercial culture	398	17.0	9.0	465	9.5	1.6
407 Wisconsin	404	17.5	4.7	394	9.6	1.7
342 United States Department of Agriculture	324	34.5	10.0	384	7.5	1.0
162 United States Department of Agriculture	395	11.5	4.5	417	8.8	1.5

[1] The figures for this treatment represent the combined results from four rows of bean plants as indicated in the plan of experiment, whereas other data given represent only the average of two similar sized rows.

It is quite uniformly indicated that the plants from the Redfield beans were affected by the wilt to a greater extent than the selected market beans and that the application of moisture to the seed previous to planting is evidently the primary cause for the stimulation of the activities of the bean wilt organism.

Observations on the roots revealed that nodules were quite generally absent and that their occasional occurrence seemed to be more closely connected to the presence of moisture in the soil than to the application of inoculating material.

CONCLUSION

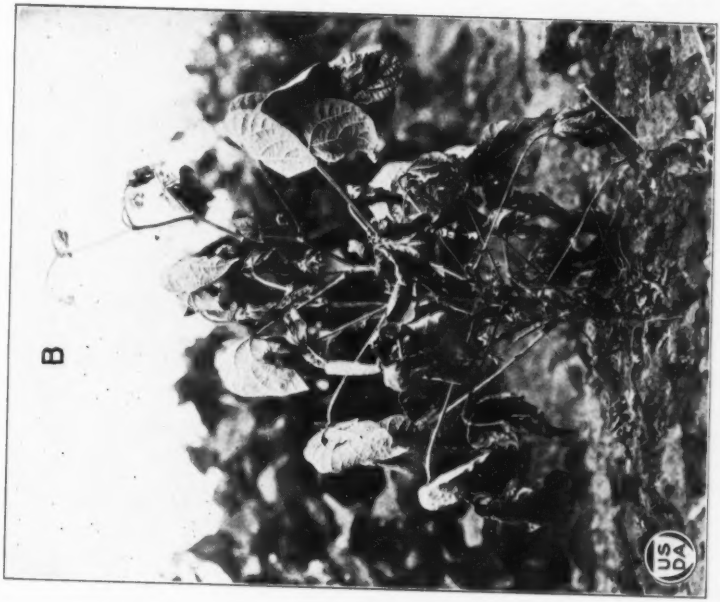
It is indicated that a slight application of moisture either in the form of water, certain culture media in the broth form or legume bacteria culture will cause a stimulation of the bean wilt disease under conditions such as obtain at Redfield, S. Dak.

If the application of legume bacteria culture to beans is necessary, it is indicated that satisfactory results may be obtained by adding this material in the form of naturally or artificially inoculated soil in a dry condition at the rate of not less than 300 pounds per acre. If only a small amount of soil is available, it may be mixed with the seed and sown with them.

This stimulation of disease organisms is apparently not produced with similar treatment under the humid conditions and soils of the Arlington Farm, Rosslyn, Va.

PLATE 1

A.—Plants in field showing symptoms of wilt beside a normal plant.
B.—A plant showing the early stages of complete destruction of life by the inroads of the wilt disease organism.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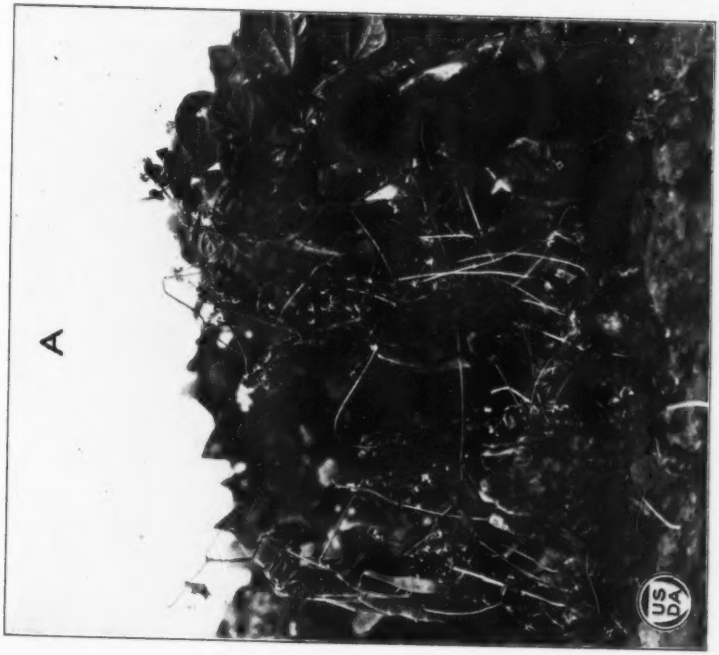
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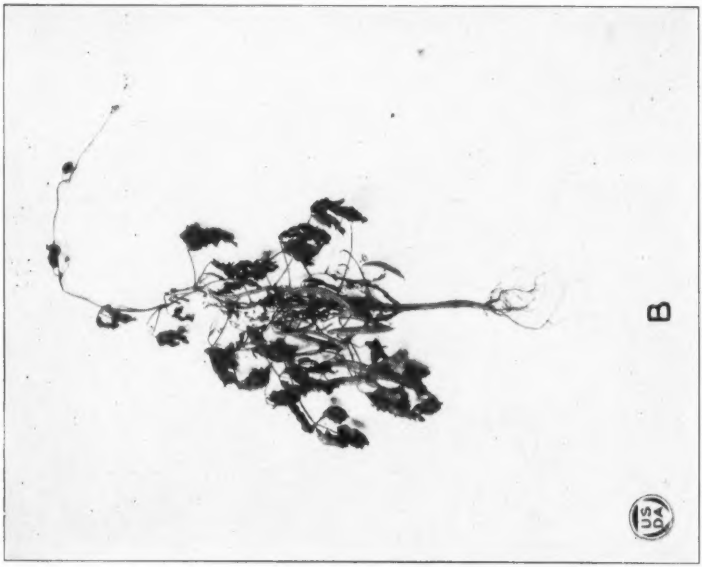
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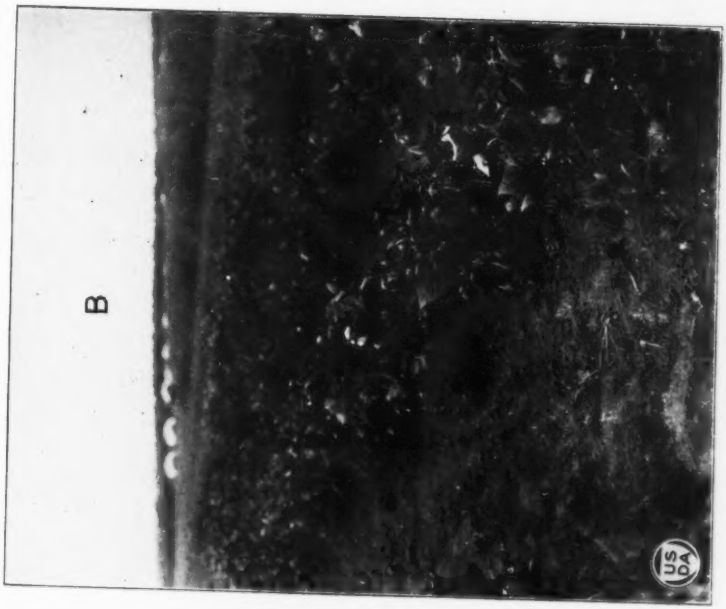
PLATE 2

A.—Plant in which disease has caused main shoot to wilt, but a side shoot is apparently unaffected.
B.—A completely destroyed bean plant which had formed pods.

43323—23——2

PLATE 3

A.—A part of a row of beans which were from seed planted dry.
B.—A part of a row of beans from seed which had been treated with Ashby broth. White cloths indicate the location of wilted specimens.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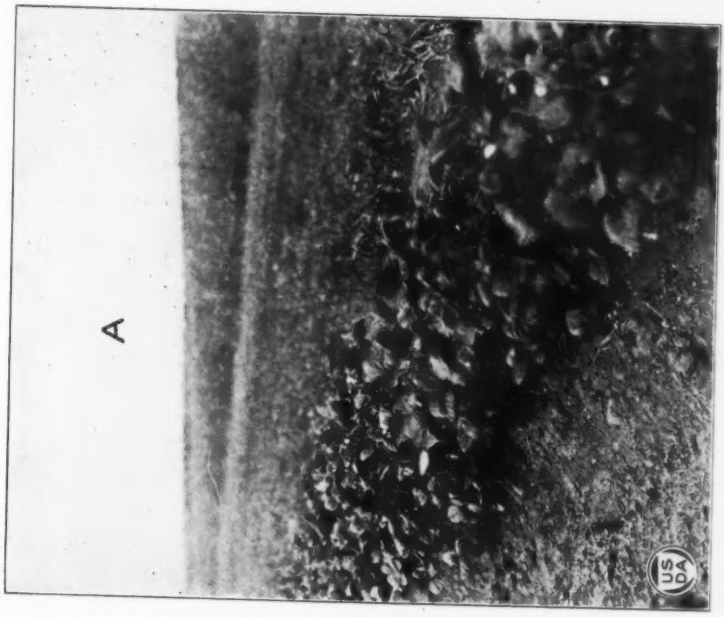
A

THE PSEUDO-ANTAGONISM OF SODIUM AND CALCIUM IN DILUTE SOLUTIONS[1]

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

The effects of calcium and of sodium ions upon living organisms are so diverse that many physiologists have advanced the idea that these ions antagonize each other at the surface of the cell. Osterhout (*6*) [2] has suggested that antagonism depends upon the production of a union of NaCl and $CaCl_2$ with some constituent of the protoplasm, and that the surface may become saturated with the antagonizing salts. Below the saturation point it is claimed that the relative proportions of the salts will be of less importance than their total concentration. In other words, it is considered that no distinctly favorable ratios exist in solutions of low concentration.

An attempt to demonstrate antagonism between Na and Ca in dilute solutions was made by Breazeale and Reed (*7*). The results of their inquiry showed an apparent antagonism existing in solutions containing 230 parts per million of total solutes. At any rate, wheat plants made somewhat better growth in ratios of $CaCl_2$ and NaCl in which Osterhout had found the greatest amount of antagonism than in other ratios.

The large amounts of calcium found by Kelley and Cummins (*5*) in citrus plants, suggested the suitability of these plants as material for the further study of this problem. In the first experiment rough-lemon (*Citrus limonia*) seedlings were grown in solutions of NaCl and $CaCl_2$ containing the following mixtures (the pure solutions each being 0.004M) 100 Na: 0 Ca; 0 Na: 100 Ca.

The distilled water used in experiments 1 and 2 was found to be calcium free as determined by evaporating 500 cc. of the water to a small volume and testing for calcium.

Liter glass jars, each containing six seedlings, were filled with culture solution on April 23. The seedlings were supported in paraffined-cork stoppers in such a way as to exclude foreign matter from the culture jars. The solutions were renewed on May 5, 13, 24, and June 1. The cultures were grown out of doors under half shade (in a lath room about 3 feet high with sides of cheesecloth) in a south exposure. On June 7 the experiment was concluded. The seedlings were momentarily dipped into and well rinsed in distilled water in order to remove adhering solution and also to remove dust from the leaves. In preliminary experiments it had been found that 0.033 gm. of dust had accumulated on 39 rough-lemon seedlings weighing 14.928 gm. green weight, 2.950 gm. dry weight, and 0.233 gm. ash.

The seedlings were freed from adhering moisture by means of filter paper, and the green weight was obtained. The seedlings were dried to constant weight at 70° C. and were then ignited in a porcelain dish at low heat. The salts in the ash were extracted with hot water and the residue

[1] Accepted for publication Sept. 2, 1922. Paper No. 97, University of California, Graduate School of Tropical Agriculture, and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 757.

and the filters were then returned to the dish, dried, and ignited. The filtrate was added to the dish, evaporated to dryness and heated at low heat to constant weight in order to obtain the weight of ash. By means of dilute HCl, practically all of the ash was brought into solution, the residue being inappreciable.

TABLE I.—*Rough-lemon seedlings grown 45 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca.....	48	21. 83	5. 90	0. 2781	0. 0105	0. 0515	0. 0376
98 Na:2 Ca.....	48	26. 16	6. 83	. 3739	. 0335	. 0587	. 0420
0 Na:100 Ca.....	49	38. 95	8. 79	. 5873	. 1259	. 0609	. 0194

The results of the first experiment (Table I) show that better plants were obtained in cultures containing calcium; in fact, both green weight and dry weight increased where the amount of calcium was increased. In the pure NaCl solution the root tips appeared stunted and brown in color. The sodium content of the plants was slightly greater in cultures containing 98 Na:2 Ca than in pure NaCl solution. In this respect the results agree with those of Reed (7), though not in regard to the relative dry weight of plants produced.

Experiment 2 was begun April 27 and concluded June 7. The solutions were renewed at the same time as were those in experiment 1. Grapefruit (*Citrus grandis*) seedlings were used.

TABLE II.—*Grapefruit seedlings grown 42 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca.....	29	36. 29	8. 34	0. 4807	0. 0124	0. 0936	0. 0547
98 Na:2 Ca.....	38	29. 62	6. 34	. 4084	. 0161	. 0850	. 0470
0 Na:100 Ca.....	42	36. 20	7. 11	. 5195	. 0646	. 0948	. 0235

From the above table it appears that the 98 Na:2 Ca ratio had no superiority over the 100 Na:0 Ca as measured by weight of plants produced. This apparent contradiction to the foregoing experiment may be due to the fact that many of the seedlings in the pure NaCl solution died and were removed. Such seedlings were discarded, for it has been shown by Johnson (4) that absorption of ions may differ appreciably in living and in dead cells. This left only the best plants in this lot; consequently we are dealing with what may be regarded as an inherently superior lot of individuals, while in the other cases we are dealing with the entire population, weak and strong.

In experiment 3, which was begun on May 4 and concluded on May 27, rough-lemon seedlings were grown in solutions of NaCl and $CaCl_2$. This

experiment was concluded early in order to avoid the possible killing of root tips. Even by so doing many of the seedlings in the solution having a ratio of 100 Na:0 Ca had dead root tips and were, therefore, discarded.

TABLE III.—*Rough-lemon seedlings grown 23 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.				
		Green weight.	Dry weight.	Ash.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca	28	23.47	4.27	0.236	0.0414	0.0272
98 Na:2 Ca	40	26.94	4.64	.273	.0532	.0225
85 Na:15 Ca	62	24.56	4.13	.263	.0446	.0157

The results of this experiment are quite at variance with those of the preceding experiments. Here the differences in green weight and dry weight are so small as to be attributable to the inherent variability of the plants rather than to differences in the composition of the solutions.

Three experiments were conducted with the same mixtures as in experiment 2, using 60 seedlings for each ratio. African sour-orange (*Citrus aurantium*), grapefruit (*C. grandis*), and St. Michael orange (*C. sinensis*) seedlings were used, the solutions being renewed at the end of two weeks. After four weeks had elapsed the experiments were discarded because the root tips of the seedlings in the solution with the ratio 100 Na:0 Ca were dead.

On June 25 rough-lemon seedlings were placed in mixtures similar to those in experiment 3. On July 8 the solutions were renewed and on July 9 the experiment was discarded. In the solution having the ratio 100 Na:0 Ca the root of nearly every seedling had become slimy and gelatinous and was dead for a considerable distance back from the root tip. The roots of the seedlings in the solutions having the ratios 98 Na:2 Ca and 85 Na:15 Ca, respectively, were bright in appearance and although small had begun to develop lateral rootlets.

The above experiments indicate that citrus seedlings can not survive many days in a solution of 230 parts per million NaCl (0.004 M; 100 Na:0 Ca). One might be led to believe that antagonism has occurred with the ratio 98 Na:2 Ca, since the seedlings are found to grow when a trace of calcium is present, whereas, in the solution with a 100 Na:0 Ca ratio they are soon stunted and shortly afterward die. Experiment 4 indicates, however, that this is an instance of calcium starvation rather than of NaCl toxicity. It was found by analyses of culture solutions that citrus seedlings do not require a large concentration of calcium provided the supply is maintained. The usual methods of chemical analysis are not sufficiently delicate to detect significant absorption differences in the brief periods required by the conductivity method of studying permeability.

Experiment 4 was carried on with St. Michael orange seedlings, which were placed in the culture jars on September 4. Each set of cultures consisted of 12 jars with 3 seedlings per jar in the first set and 2 seedlings per jar in the remainder.

The first culture solution was that employed by Hoagland (*3*). The cultures were still growing on November 1. The second set of cultures

was grown in Hoagland's nutrient solution in which the calcium had been replaced by potassium; 500 cc. of this solution was evaporated to a small volume and was then tested and found to be calcium-free. Within 3 days after the experiment was begun, the root tips of the seedlings in the calcium-free cultures were becoming brown and failed to show any evidence of growth. After 7 to 10 days the root tips of most of the seedlings were becoming slimy and gelatinous. At the end of 2 weeks most of the roots appeared to be dead. The full amount of calcium of Hoagland's nutrient solution was then added in the form of calcium nitrate to six of the jars. Within a few days many of the seedlings began to recover, although several of the seedlings in advanced stages of decomposition failed to respond to the addition of the calcium nitrate.

In Plate 1, A, B, are shown various stages in the progressive killing of the primary root, beginning with the root tip and proceeding toward the cotyledons. Plate 1, B, shows some of the roots of Plate 1, A, somewhat enlarged. (The material closely held by the roots is composed of small pieces of sphagnum from the germination box.) It will be seen that when calcium was added to the solution, laterals were developed, the lowermost lateral always sharply separating the living from the dead portions of the primary root. Frequently in the absence of calcium the primary root was unable to grow. In such cases, when calcium was added, a lateral rootlet was produced immediately back of the root tip and this lateral then assumed the position of the primary root (Plate 1, A, B). One of the most characteristic effects of a lack of calcium is the gelatinization of the surface layers of the root. This process is most active in the apical region of the root and progressively decreases in intensity toward the upper portion. As time goes on the gelatinization proceeds inward until finally the root becomes translucent.

In the initial stages of this process of gelatinization, recovery is possible if a suitable amount of a calcium salt is added. The root then regains its firm white appearance and functions properly. In advanced stages of gelatinization recovery does not follow the addition of a calcium salt, although new laterals may be produced from the upper portion of the root. Herbst (*2*), Hansteen Cranner (*1*), and others have observed a similar gelatinization in other organisms in the absence of calcium and likewise have observed recovery when calcium salts were added.

There is thus evidence that the stunted appearance of roots in pure NaCl solutions above discussed is not due to the toxicity of NaCl but to the lack of calcium. Further study of this factor was made in which more concentrated solutions of NaCl, having toxic properties, were used. Seedlings placed in Hoagland's solution minus calcium failed to develop although it contained but 30 parts per million NaCl. They speedily recovered and grew when $CaCl_2$ was added. Another series in calcium-free Hoagland's solution plus 1,000 parts per million NaCl likewise failed to develop. When $CaCl_2$ was added to this latter solution the plants made good growth in spite of the amount of NaCl present.

It appears, therefore, that the harmful effect of 100 Na:0 Ca in dilute solutions is not due to the lack of ions which antagonize the sodium, but rather to calcium starvation, and that the result is conspicuous in the case of a plant like citrus which is extremely sensitive to calcium. In solutions containing only 0.004 M. NaCl, it seems improper to speak of a toxic action upon the roots. (Cf. True, *8*.)

In the study of the antagonism of Na and Ca with citrus seedlings it seems impossible to utilize solutions as dilute as those commonly employed as nutrients on account of the starvation which results when the calcium is reduced to extremely low concentrations. In other words, the assumption that no distinctly favorable ratios exist in solutions of low concentration is supported.

SUMMARY

Citrus seedlings grown in absence of calcium soon show injury to the root system, as evidenced by the gelatinization of the superficial layers and the ultimate death of the root. The tops, however, may not show the effect for some time after the injury to the roots has become severe.

If the injury has not progressed too far, the addition of calcium to cultures in which the roots are gelatinized induces the production of lateral rootlets the lowermost of which definitely delimits the dead from the living portion of the root.

The varieties of citrus here studied are known to possess a marked capacity for the absorption of calcium ions. Hence in very dilute solutions the amounts present may be too far below the equilibrium point within the plant to avoid a condition of starvation. In such cases we are dealing with the phenomenon of starvation rather than that of antagonism. These experiments with citrus seedlings have not demonstrated the existence of antagonism between sodium and calcium when the plants are grown in very dilute solutions.

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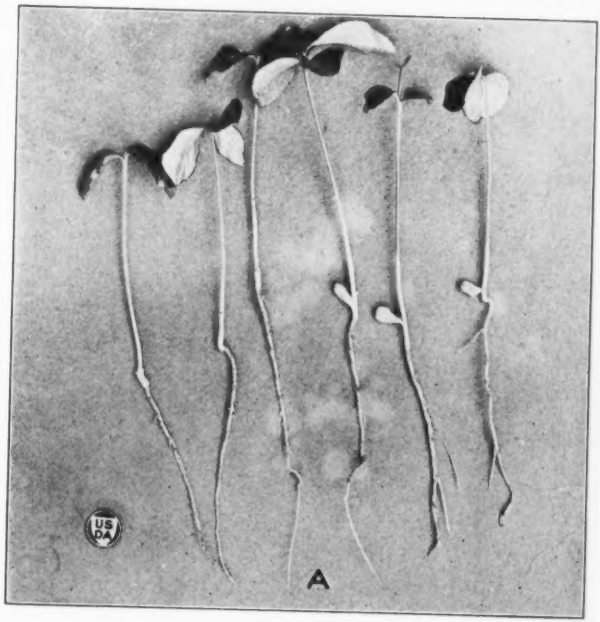
PLATE 1

A.—Orange seedlings grown in calcium-free Hoagland's solution. At the end of two weeks the full amount of calcium of Hoagland's solution was added in the form of calcium nitrate. The point at which recovery occurred is seen on each root.

B.—Showing the point of recovery of roots of orange seedlings (Pl. 1, A) when calcium is added to the solution after two weeks' exposure to calcium-free Hoagland's solution.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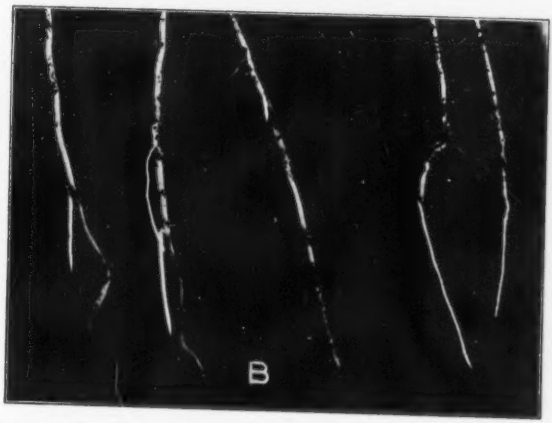
B

INFLUENCE OF THE HYDROGEN-ION CONCENTRATION ON THE GROWTH AND FIXATION OF NITROGEN BY CULTURES OF AZOTOBACTER [1]

By P. L. GAINEY, *Professor of Bacteriology*, and H. W. BATCHELOR, *Laboratory Assistant in Bacteriology, Kansas Agricultural Experiment Station*

Investigations in this laboratory have shown that the presence or absence of Azotobacter in natural soils is very closely associated with, if not dependent upon, the absolute reaction of the soil solution (*4*).[2] It has also been shown that in the laboratory the presence of this group of organisms in a soil can be controlled by varying the hydrogen-ion concentration of the soil (*5*).

In connection with these investigations it seemed desirable to study the influence of the hydrogen-ion concentration of laboratory culture media upon the growth and nitrogen fixing ability of pure cultures of Azotobacter.[3]

In their original investigations on Azotobacter, Beijerinck and Van Delden (*1*) failed to secure appreciable fixation of nitrogen by pure cultures. On the other hand, Lipman (*6*) experienced no difficulty in securing marked fixation by pure cultures, and he presents data to show that the ability of pure cultures to fix nitrogen in the media usually employed for their culture depended upon the neutralization of the acidity arising from the potassium phosphate present. Lipman further demonstrated that, quantitatively, the nitrogen fixed by pure cultures was inversely proportional to the titratable acidity of the media, and called attention to the probability that Beijerinck's failure to secure nitrogen fixation was due to the unfavorable reaction of the medium employed. Since Lipman's work appeared, it has been universally accepted that these organizations will not function in a high concentration of acid.

With regard to the effect of different degrees of acidity, or hydrogen-ion concentrations, upon the growth of pure cultures of this group of organisms little information has been published. Fred (*3*) observed the growth of two different cultures in a medium of varying hydrogen-ion concentrations and noted no growth at P_H 6.4 to 6.6, while growth occurred at P_H 6.6 to 6.8. On the other hand, when grown in a medium of P_H 7.2 the final hydrogen-ion concentration was found to be P_H 5.1. As already mentioned, previous investigations in this laboratory indicated that the hydrogen-ion concentration of the soil solution is the major factor in controlling the presence of this group of organisms in soils. Also, that the maximum concentration of hydrogen ions tolerated by this group of

[1] Accepted for publication Oct. 16, 1922. Contribution No. 50, Department of Bacteriology, Kansas Agriculture Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 767.

[3] The term "pure culture" is here used in a restricted sense. Owing to the extreme morphological variations exhibited by this group of organisms it is frequently very difficult to ascertain with certainty the purity of a given culture. All cultures used in these experiments conformed to the description given in the text of this paper.

organisms appear to be very close to P_H 5.9 to 6.0. It seemed of interest, therefore, to ascertain the effect of varying the hydrogen-ion concentration of laboratory media upon the activity of a number of strains of Azotobacter.

METHODS EMPLOYED

Crude cultures of Azotobacter were prepared from a number of soils by inoculating a mannite or dextrose cultural solution and incubating until a characteristic Azotobacter film developed. Portions of these films were then streaked upon mannite or dextrose agar and the streaking process repeated from isolated colonies until all colonies developing were similar and yielded, when stained, only typical Azotobacter cells. No effort was made to identify the various cultures.

The medium employed had the following composition: Monobasic potassium phosphate, 0.2 or 5.0 gm.; magnesium sulphate, 0.2 gm.; sodium chlorid, 0.5 gm.; ferric chlorid, 2 drops of a 10 per cent solution; dextrose or mannite, 20.0 gm.; distilled water 1,000 cc. Flasks of this medium, slightly alkaline to brom-thymol-blue, were inoculated from an agar streak culture and aerated vigorously at 28° C. until heavily clouded (two to four days). The cultures were again stained and examined, and all flasks showing morphological forms other than typical Azotobacter were discarded. One to five per cent of such a culture was used as an inoculum.

The quantity of sterile media necessary to set up an experiment was prepared and inoculated. Fifty cubic centimeter quantities were measured aseptically into carefully washed sterile 300 cc. Erlenmeyer flasks. The quantity of NaOH or HCl necessary to adjust the 50 cc. of media to approximately the desired reaction was determined and this quantity, previously sterilized, was carefully added to four flasks. The hydrogen-ion concentration of the contents of one of these flasks was immediately determined and recorded as the initial reaction. Hydrogen-ion determinations were made colorimetrically, a control being occasionally run electrometrically. A small quantity of formaldehyde was added to a second flask to act as a control with which to compare growth and nitrogen fixation. The remaining two cultures were incubated at 28° C. for two weeks. The quantity of acid or alkali required to produce a given change in reaction varied only slightly from time to time.

In the earlier experiments, where only 0.02 per cent KH_2PO_4 was used, the buffer index was very low and necessitated the use of very dilute (N/80) NaOH in adjusting the reaction. Where 0.5 per cent KH_2PO_4 was used (experiments 16 to 20 inclusive) the buffer index was much higher, requiring approximately ten times as much acid or alkali to produce a given change in reaction, and N/10 NaOH was used to correct the reaction.

During incubation the cultures were examined at frequent intervals and the presence or absence of growth recorded. The formaldehyde produced a slight change in the physical appearance of the medium in the control flasks and rendered it somewhat difficult to detect very slight changes in the appearance of cultures. Where it was impossible to determine definitely whether growth had taken place it has been indicated in Tables I and IV with a question mark.

After two weeks' incubation a final examination for growth was made, the hydrogen-ion concentration of the cultures recorded, and the total nitrogen present determined by the Kjeldahl method. The quantity of

nitrogen in the control flasks and in the cultures where no growth was visible was very small and, as will be observed in Table II, the experimental error for the controls was large. In examining the data relative to the quantity of nitrogen fixed this experimental error must be taken into consideration, otherwise erroneous conclusions are likely to be drawn. In the data recorded in Tables II and IV the quantity of nitrogen present in controls has been deducted from that present in the inoculated flasks and only the net gain recorded. Nitrogen determinations were made on at least four control flasks to determine the probable error where only small quantities of nitrogen were present.

INFLUENCE OF REACTION UPON GROWTH

In Table I data are recorded relative to the influence of the hydrogen-ion concentration of the culture medium upon the growth of several strains of Azotobacter. A study of these data will show that the maximum acidity permitting appreciable growth to take place is very close to P_H 5.9. There is only one instance where definite growth is recorded in a higher degree of acidity (experiment 15, P_H 5.8) and the duplicate of this culture failed to show growth. Another strain, 12–323–6, not cultured at P_H 5.9 in the experiment here recorded grew at P_H 5.9 in an unrecorded experiment. All cultures showed some growth at P_H 5.9–6.0. The amount of growth that took place at P_H 5.9 was in most instances very slight, and growth increased as the acidity decreased until P_H 6.1 to 6.3 was reached. At this reaction growth appeared in most instances to be as vigorous as at lower hydrogen-ion concentrations. Cultures 407–5 and 449 did not grow as abundantly as did the other cultures, even when the reaction was favorable. These two cultures failed to produce a surface film, and when growth is compared with nitrogen fixation it will be noted that they also fixed small quantities of nitrogen.

TABLE I.—*The influence of the hydrogen-ion concentration of culture media upon the growth of Azotobacter*

Experiment No.	Culture.	Initial reaction expressed as P_H (growth records for 2 weeks).																		
		Below 5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.2	Above 7.6	$CaCO_3$
6	426–21	−	−	.....	−	−	+	++	++++	++++	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		−	−	.....	−	.....	+	++	++++	++++	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	C	−	.....	−	.....	−	.....	+	++	.....	.....	++++	++++	.....	.....	.....	.....	.....	.....	.....
		−	.....	−	.....	−	.....	+	++	.....	.....	++++	++++	.....	.....	.....	.....	.....	.....	.....
9	19–399	.....	−	−	.....	.....	−	.....	.....	+	++++	.....	.....	++++	.....	.....	.....	.....	.....	.....
		.....	−	−	.....	.....	+	.....	.....	+	++++	.....	.....	++++	.....	.....	.....	.....	.....	.....
11	426–21	.....	.....	−	.....	.....	.....	+	+	.....	.....	++++	++++	++++	.....	++++	++++	.....	.....	.....
		.....	.....	?	.....	.....	+	+	++++	.....	.....	++++	++++	++++	.....	++++	++++	++++	++++	.....
12	40–399	−	.....	?	.....	.....	+	++	.....	.....	++++	.....	++++	.....	.....	.....	.....	++++	++++	.....
		−	.....	?	.....	.....	+	++	.....	.....	++++	.....	++++	.....	.....	.....	.....	++++	++++	.....
13	12–323–6	−	.....	−	.....	.....	.....	+	.....	++	++++	.....	.....	++++	.....	.....	.....	++++	++++	.....
		−	.....	?	.....	.....	.....	+	.....	++	++++	.....	.....	++++	.....	.....	.....	++++	++++	.....
14	C	.....	.....	?	?	.....	+	+	+	.....	.....	.....	++++	.....	.....	.....	.....	++++	++++	.....
		.....	.....	?	?	.....	+	+	+	.....	.....	.....	++++	.....	.....	.....	.....	++++	++++	.....
15	40–399	.....	−	−	−	−	+	++	++	.....	.....	.....	++	.....	++	++++	.....	.....	.....	++++
		.....	−	−	−	+	++	++	++	.....	.....	.....	++++	.....	++	.....	.....	.....	.....	.....
16	407–5	−	.....	−	−	−	?	+	.....	.....	.....	+	+	.....	.....	.....	.....	.....	.....	.....
		−	.....	?	−	−	?	+	.....	.....	.....	+	+	.....	.....	.....	.....	.....	.....	.....
17	14–399–6	−	.....	.....	?	.....	+	.....	+	.....	++++	.....	++++	.....	.....	++++	.....	.....	.....	++++
		−	.....	.....	?	.....	+	.....	+	.....	++++	.....	++++	.....	.....	++++	.....	.....	.....	++++
18	E	−	.....	.....	−	.....	+	++	++	++++	++++	.....	.....	.....	.....	++++	.....	.....	.....	.....
		−	.....	.....	−	.....	+	++	++	++++	++++	.....	.....	.....	.....	++++	.....	.....	.....	.....
19	407–5	−	.....	.....	.....	−	−	.....	?	.....	+	++	.....	.....	.....	++	.....	.....	.....	.....
		−	.....	.....	.....	−	+	.....	?	.....	+	++	.....	.....	.....	++	.....	.....	.....	.....
20	499	−	−	.....	−	.....	−	+	+	+	.....	.....	.....	.....	.....	++	.....	.....	.....	++++
		−	−	.....	−	.....	?	?	+	+	.....	.....	.....	.....	.....	++	.....	.....	.....	++++

− No visible growth.
? Questionable growth.
+ Slight but distinct growth.
++ Good growth, but no film.
++++ Heavy growth and film formation.

INFLUENCE OF REACTION UPON NITROGEN FIXATION

Data relative to the influence of the hydrogen-ion concentration of the culture medium upon the fixation of nitrogen by cultures of Azotobacter are recorded in Table II. In examining these data the rather large experimental error for the controls of the individual experiments should be noted. In no instance is there recorded a definite fixation of nitrogen in a hydrogen-ion concentration greater than P_H 5.9. The data for P_H 5.9, 6.0, and 6.1 are inconclusive, the quantities of nitrogen fixed, if any, being small. At P_H 6.2 and above definite fixation occurred, the maximum in most experiments being recorded at P_H 6.3 to 6.5. Vigorous fixation of nitrogen appeared to be associated with the growth of a definite film on the surface of the medium.

TABLE II.—*The influence of the hydrogen-ion concentration of culture media upon the fixation of nitrogen by Azotobacter*

Experiment No.	Culture.	Initial reaction expressed as P_H. nitrogen fixed per culture of 50 cc. media (mgm.).																		Probable error controls.
		Below 5.4.	5.5.	5.6.	5.7.	5.8.	5.9.	6.0.	6.1.	6.2.	6.3.	6.4.	6.5.	6.6.	6.8.	6.9.	7.2.	Above 7.6.	$CaCO_3$.	
11	426–21	.....	.....	0.08	.....	.....	.....	0.34	0.17	.....	.....	4.12	2.94	4.81	.....	5.18	4.84	.....	.....	±0.51
		.....	.....	0.00	.....	.....	0.43	0.00	1.81	.....	.....	4.75	2.85	5.44	.....	5.27	.....	.....	.....	±0.51
12	40–399	0.00	.....	0.00	.....	.....	0.83	1.11	.....	.....	3.79	.....	3.70	.....	.....	.....	.....	3.97	.....	±0.95
		0.37	.....	0.74	.....	.....	0.00	0.00	.....	.....	4.06	.....	4.62	.....	.....	.....	.....	3.98	.....	±0.95
13	12–232–6	0.00	.....	0.18	.....	.....	.....	1.02	.....	1.20	2.14	.....	.....	4.43	.....	.....	.....	4.62	.....	±0.46
		0.00	.....	0.00	.....	.....	.....	0.18	.....	1.48	4.43	.....	.....	3.79	.....	.....	.....	4.53	.....	±0.46
14	C	.....	.....	0.51	0.35	.....	1.35	0.51	1.16	.....	.....	.....	5.31	.....	.....	.....	.....	5.59	.....	±1.01
		.....	.....	0.32	0.69	.....	0.60	0.60	0.10	.....	.....	.....	5.78	.....	.....	.....	.....	3.66	.....	±1.01
16	407–5	0.00	.....	0.28	0.19	0.47	.....	0.47	.....	.....	.....	0.85	2.48	.....	.....	.....	.....	.....	.....	±0.45
		0.28	.....	0.19	0.09	0.09	.....	0.57	.....	.....	.....	0.66	.....	.....	.....	.....	.....	.....	.....	±0.45
17	14–399–6	0.00	.....	.....	0.21	.....	0.31	.....	0.62	.....	0.93	.....	4.64	.....	.....	5.37	.....	.....	5.59	±0.43
		0.10	.....	.....	0.82	.....	0.21	.....	0.41	.....	0.72	.....	5.06	.....	.....	4.75	.....	.....	4.73	±0.43
18	E	0.00	.....	.....	0.17	.....	0.17	1.18	0.41	1.43	1.12	.....	.....	.....	.....	1.84	.....	.....	.....	±0.77
		0.00	.....	.....	loss	.....	0.20	1.63	0.92	1.22	1.43	.....	.....	.....	.....	1.63	.....	.....	.....	±0.77
19	407–5	0.00	.....	.....	.....	0.00	0.00	.....	0.32	.....	0.22	0.65	.....	.....	.....	2.38	.....	.....	.....	±0.05
		0.14	.....	.....	.....	0.00	0.47	.....	0.00	.....	0.54	0.76	.....	.....	.....	1.73	.....	.....	.....	±0.05
20	499	0.56	0.00	.....	0.00	.....	0.00	0.10	1.07	0.73	.....	.....	.....	.....	.....	1.50	.....	.....	4.28	±0.64
		0.31	0.21	.....	0.21	.....	0.63	1.58	1.26	1.89	.....	.....	.....	.....	.....	3.78	.....	.....	4.55	±0.64
Average....		0.12	0.11	0.23	0.27	0.14	0.40	0.66	0.77	1.33	1.94	1.97	4.15	4.62	2.87	5.22	4.84	4.39	4.79	±0.27

CHANGES IN REACTION PRODUCED BY GROWTH

The initial and final reactions of the cultures are recorded in Table III. The medium used in all experiments below 16 contained only 0.02 per cent KH_2PO_4 and possessed a very low buffer index. The quantity of N/1 base or acid necessary to produce a change of 0.1 P_H in 100 cc. of this medium was only approximately 0.006 cc. In these experiments some rather marked changes in reaction were recorded. The changes sometimes indicated the production of acid, at other times the production of basic compounds. However, when calculated in terms of total acid or base the quantities are insignificant.

TABLE III.—*The influence of the growth of Azotobacter in culture media upon the reaction of the media*

Experiment No.	Culture.	Initial reaction expressed as P_H, after 2 weeks' growth.																		
		Below 5.4.	5.5.	5.6.	5.7.	5.8.	5.9.	6.0.	6.1.	6.2.	6.3.	6.4.	6.5.	6.6.	6.7.	6.8.	6.9.	7.2.	Above 7.6.	Per cent KH_2PO_4.
		Less than-																		
6	426–21	5.4—	5.4—	.....	5.6	5.8	5.8	5.9	6.1	6.0	.....	5.8	.....	.....	.....	.....	.....	.....	.....	[a] 0.02
		5.4—	5.5	.....	5.7	5.8	5.9	6.0	6.1	6.2	.....	6.4	.....	.....	.....	.....	.....	.....	.....	.02
7	12–232–6	.....	5.4	.....	5.8	.....	5.9	.....	.....	.....	6.1	.....	6.1	.....	6.8	.....	.....	.....	.....	.02
		.....	5.4	.....	5.8	.....	5.9	.....	.....	.....	6.1	.....	6.3	.....	6.8	.....	.....	.....	.....	.02
8	C	.....	.....	.....	.....	.....	.....	5.8	6.3	.....	.....	6.3	6.6	.....	.....	.....	.....	.....	.....	.02
		.....	.....	.....	.....	.....	.....	6.0	5.9	.....	.....	6.4	6.6	.....	.....	.....	.....	.....	.....	.02
1	426–21	.....	.....	5.4—	.....	.....	5.4—	5.4—	5.4—	.....	.....	6.7	5.4—	7.3	.....	.....	7.3	7.4	.....	.02
		.....	.....	5.4—	.....	.....	5.6	5.4—	5.5	.....	.....	6.7	6.4	7.3	.....	.....	7.5	.....	.....	.02
11	40–399	5.4—	.....	5.7	.....	.....	5.7	5.8	.....	.....	6.5	.....	5.6	.....	.....	.....	.....	.....	5.7	.02
		5.6	.....	5.7	.....	.....	5.7	5.7	.....	.....	6.5	.....	6.4	.....	.....	.....	.....	.....	5.9	.02
13	12–232–6	5.4—	.....	5.5	.....	.....	.....	5.4—	.....	5.4—	6.4—	.....	.....	7.3	.....	.....	.....	.....	7.5	.02
		5.4—	.....	5.4	.....	.....	.....	5.4	.....	5.4—	5.4—	.....	.....	6.5	.....	.....	.....	.....	7.2	.02
14	C	.....	.....	5.4	5.4	.....	5.4	5.7	5.8	.....	.....	.....	6.8	.....	.....	.....	.....	.....	7.0	.02
		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.7	.....	.....	.....	.....	.....	7.2	.02
16	407–5	5.4—	.....	5.8	5.8	.....	5.9	6.1	.....	.....	.....	6.4	6.4	.....	.....	.....	.....	.....	.....	[b] 0.50
		5.4—	.....	5.7	5.9	.....	5.9	6.1	.....	.....	.....	6.4	6.5	.....	.....	.....	.....	.....	.....	.50
17	14–399–6	5.4—	.....	.....	5.6	.....	5.9	.....	6.1	.....	6.3	.....	6.7	.....	.....	6.7	.....	.....	.....	.50
		5.4—	.....	.....	5.8	.....	5.8	.....	6.0	.....	6.3	.....	6.6	.....	.....	6.7	.....	.....	.....	.50
18	E	5.4—	.....	.....	5.8	.....	5.9	6.4	6.1	6.1	6.3	.....	.....	.....	.....	6.6	.....	.....	.....	.50
		5.4—	.....	.....	5.6	.....	5.9	6.4	6.1	6.2	6.2	.....	.....	.....	.....	6.6	.....	.....	.....	.50
19	407–5	5.4—	.....	.....	.....	5.6	5.8	.....	6.0	.....	6.4	6.4	.....	.....	.....	6.8	.....	.....	.....	.50
		5.4—	.....	.....	.....	5.8	5.8	.....	6.0	.....	6.1	6.4	.....	.....	.....	6.8	.....	.....	.....	.50
20	449	5.4—	5.4—	.....	5.4—	.....	5.6	5.9	6.0	6.1	.....	.....	.....	.....	.....	6.8	.....	.....	.....	.50
		5.4—	5.4—	.....	5.7	.....	5.8	5.9	6.1	5.4—	.....	.....	.....	.....	.....	6.8	.....	.....	.....	.50

[a] Media containing 0.02 per cent KH_2PO_4 required only 0.006 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.
[b] Media containing 0.50 per cent KH_2PO_4 required 0.05 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.

These results led to the conclusion that no satisfactory data as to changes in reaction could be secured in a medium so poorly buffered. The quantity of KH_2PO_4 was accordingly increased to 0.5 per cent. Higher concentrations of phosphate were sometimes found to be toxic. This increase in phosphate increased the buffer effect approximately ten times, the media containing 0.5 per cent requiring 0.05 cc. N/1 NaOH to effect a change of 0.1 P_H in 100 cc. The buffer effect was still low, but an examination of experiments 16 to 20 will show that changes in P_H produced by the growth of the various cultures were practically eliminated. If acid or basic metabolic by-products are produced under these experimental conditions the quantities are inappreciable.

Fred (3) recorded a change in P_H from 7.2 to 5.1. The media employed by him, however, contained only 0.02 per cent phosphate, and the actual quantity of acid necessary to produce the recorded change in P_H was probably very small. Stoklasa (7) recorded the production of as high as 3.3 cc. N/1 acid per 100 cc. media. This quantity would have produced very marked changes in the P_H of our media. However, the purity of Stoklasa's cultures has been questioned by Bonazzi (2).

In Table IV are recorded in parallel columns the initial reaction, growth, final reaction, and milligrams of nitrogen fixed for four experiments. This table is included in order that these various factors may be compared one with the other without the necessity of examining several tables. In figure 1 the influence of the reaction on nitrogen fixation is shown graphically.

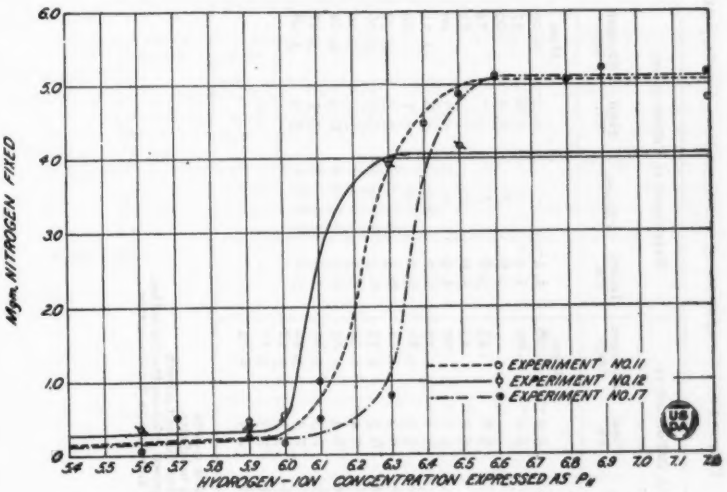

FIG. 1.—Effect of hydrogen-ion concentration on nitrogen fixation.

[a] Media containing 0.02 per cent KH_2PO_4 required only 0.006 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.
[b] Media containing 0.50 per cent KH_2PO_4 required 0.05 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.

TABLE IV.—*Comparison of initial reaction, growth, final reaction, and nitrogen fixed by four cultures of Azotobacter*

Experiment 11, Culture 426–21.				Experiment 12, Culture 40–399.				Experiment 17, Culture 14–399–6.				Experiment 18, Culture E.			
Initial Ps.	Growth.	Final Ps.	Nitrogen fixed.	Initial Ps.	Growth.	Final Ps.	Nitrogen fixed.	Initial Ps.	Growth.	Final Ps.	Nitrogen fixed.	Initial Ps.	Growth.	Final Ps.	Nitrogen fixed.
			Mgm.				*Mgm.*				*Mgm.*				*Mgm.*
5.6	—	5.4—	0.08	5.4—	—	5.4—	0.00	5.4—	—	5.4—	0.00	5.4—	—	5.4—	0.00
5.6	?	5.4—	.00	5.4—	—	5.6	.37	5.4—	—	5.4—	.10	5.4—	—	5.4—	.00
5.9	.....	.....	.....	5.6	?	5.7	.00	5.7	?	5.6	.21	5.7	—	5.8	.17
5.9	?	5.6	.43	5.6	?	5.7	.74	5.7	?	5.8	.82	5.7	—	5.6	.00
6.0	?	5.4—	.43	5.9	++	5.7	.83	5.9	+	5.9	.31	5.9	++	5.9	.17
6.0	?	5.4—	.00	5.9	++	5.7	.00	5.9	+	5.8	.21	5.9	+++	5.9	.20
6.1	?	5.4—	.17	6.0	+++	5.8	1.11	6.1	+	6.1	.62	6.0	+++	6.4	1.12
6.1	+	5.5	1.81	6.0	+++	5.7	.00	6.1	+	6.0	.41	6.0	+++	6.4	1.63
6.4	+++	6.7	4.12	6.3	+++	6.5	3.79	6.3	+++	6.3	.93	6.0	+++	6.1	.41
6.4	+++	6.7	4.75	6.3	+++	6.5	4.00	6.3	+++	6.3	.73	6.1	+++	6.1	.92
6.5	+++	6.6	2.94	6.5	+++	6.6	3.70	6.5	+++	6.7	4.64	6.1	+++	6.1	1.43
6.5	+++	5.4—	2.85	6.5	+++	6.4	4.62	6.5	+++	6.6	5.06	6.2	+++	6.2	1.22
6.6	+++	6.4	4.81	7.6	+++	5.7	3.97	6.8	+++	6.7	5.37	6.3	+++	6.3	1.12
6.6	+++	7.3	5.44	7.6	+++	5.9	3.98	6.8	+++	6.7	4.75	6.3	+++	6.2	1.43
6.9	+++	7.3	5.18	.....	.....	.....	.....	Ca CO_3	+++	.....	5.59	6.8	+++	6.6	1.84
6.9	+++	7.5	5.27	.....	.....	.....	.....	Ca CO_3	+++	.....	4.73	6.8	+++	6.6	1.63
7.2	+++	7.4	4.84	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

— No visible growth.
? Growth indefinite.
+ Slight but distinct growth.
++ Good growth, with no film formation.
++++ Heavy growth with film formation.

SUMMARY

The data presented in this paper point very definitely to a limiting hydrogen-ion concentration of P_H 5.9 to 6.0 for the various cultures of Azotobacter employed when grown under the conditions of these experiments. Vigorous growth and nitrogen fixation took place at P_H 6.1 to 6.5, the optimum P_H for nitrogen fixation apparently being somewhat higher than the optimum for growth. Very slight, if any, changes in the reaction of the media are produced by the growth of the various strains of Azotobacter studied, indicating the production of inappreciable quantities of acid or basic metabolic by-products.

The data for pure cultures here presented agree very closely with our findings in soil and tend to substantiate former conclusions that this group of organisms will not exist and function in soils, the hydrogen-ion concentration of which is greater than P_H 5.9 to 6.0.

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43323—23——3

SUNFLOWER INVESTIGATIONS[1]

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INTRODUCTION

The value of sunflowers for a silage crop in the Pacific Northwest has become quite well established. Their resistance to drought and early frosts, and the fact that they yield a large tonnage of green material per acre, justify a more complete knowledge of the proper spacing of plants in the row and the best stage of cutting the plant for silage purposes. With these facts in mind, an investigation was planned which would yield data on the proper method of planting and the proper time of harvesting.

The composition of the sunflower plants was first studied when harvested at various stages during growth, and when grown under two different spacings, namely, 4 to 8 inches, and 36 inches, apart in the row. The sunflower plants grown under the two systems of planting were compared for the percentages of leaves, stalks, stems, and flowers, and analyses were made of composite samples of each of these plant parts. Not only was the composition of the sunflowers determined in these two systems of planting and at various stages of maturity, but also the composition of the silage made from the sunflowers cut at each of these stages of maturity and from each of the two different spacings of plants in the row.

PREVIOUS WORK

Shaw and Wright,[2] of the United States Department of Agriculture, published data on the composition of sunflowers grown at the Dairy Division Experiment Farm at Beltsville, Md. Their investigation included the composition of sunflowers cut at seven different stages of maturity.

Blish,[3] of the Montana Experiment Station, has recently published data on the effect on the composition and quality of silage of cutting sunflowers at different stages of growth. All results showed that silage of good quality resulted from sunflowers cut at the different stages of maturity selected by him. The problems, then, that present themselves in Idaho are: First, what is the most favorable stage of maturity for harvesting the sunflowers for silage purposes, and, second, what is the proper distance apart of planting in order that the maximum feeding value may be secured?

EXPERIMENTAL WORK

The sunflowers used in this investigation were grown at the Idaho State Experiment Station at Moscow, Idaho, in the summer of 1920, by officials of the agronomy department, who kindly allowed us to sample the

[1] Accepted for publication Oct. 16, 1922.

[2] SHAW, R. H., and WRIGHT, P. A. A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH. *In* Jour. Agr. Research, v. 20, p. 787–793. 1921. Literature cited, p. 792–793.

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sunflowers at all stages of maturity. Additional work on the 1921 crop of sunflowers is included later in this paper.

The plants were grown from seed of the Giant Russian variety on Palouse silt loam soil. The plants grew luxuriantly, many of the largest of them reaching a height of from 10 to 12 feet.

STAGES OF MATURITY SELECTED

In selecting the proper stages at which to sample the sunflowers considerable difficulty was encountered in choosing definite stages of growth, since the sunflower plant does not offer any definite and sharply defined differences at any time during its growth. After due consideration, the stages were arbitrarily chosen in terms of budding and flowering in the early period of growth and, later on, of the degree of hardness of the seeds in the main or top flower. Five stages were selected, as follows: First, when the first bud was appearing on the top of the plant; second, when the first flower was about 3 inches in diameter but no seed had developed; third, just before the seeds of the first flower were in the dough stage; fourth, when the seeds of the first flower were well into the dough stage and the rays were just beginning to fall; fifth, when the seeds of the first flower were quite hard and its rays had fallen.

METHOD OF SAMPLING

Representative samples were secured by collecting a number of plants of uniform development and weighing and measuring each plant; the average was then recorded. Composite samples of sufficient size were made by cutting in a small silage cutter the constituent plants and then mixing thoroughly. A portion of each sample was dried in an electric oven to secure the percentage of moisture. A second portion was air dried and reserved for the approximate analysis. The remaining portion was used for making silage. For this purpose quart milk bottles were tightly filled and each stoppered with a rubber stopper containing a bent glass tube which had its outlet in a beaker of mercury. This arrangement allowed the fermentation gases to escape but prevented air from gaining access to the silage. This procedure was found to be very satisfactory and in every case resulted in a good quality of silage.

DIFFERENT SPACINGS OF PLANTS

In the early stages of growth, when the plants were about 6 inches high, the plants were thinned in order to secure data on the yield and composition of sunflowers when grown at various distances apart in the row.

In order to secure chemical data, two distances apart were chosen, which represented as nearly as possible the extremes of planting and also the two extreme types of sunflowers.[4] The two types selected were plants grown 36 inches apart and plants grown from 4 to 8 inches apart. The former system resulted in plants with an abundance of leaves and flowers on one large stalk, while the latter resulted in rather tall, spindly stalks with much smaller leaves and flowers. The data on the composition of sunflowers and sunflower silage are given in Table I.

[4] The spacings of plants intermediate between 4 inches to 8 inches and 36 inches were not included in this investigation, as the analytical work would have been enormously increased, and it was not certain that the results would show differences sufficient to warrant the additional analyses.

TABLE I.—*Composition of sunflowers and sunflower silage (1920)*

Stage of growth.	Average height.		Average weight.		Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extracted.	Crude fiber.	Nitrogen-free extract.	Ash.	Type of material analyzed.
	Ft.	*in.*	*Lbs.*	*oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Budding	8	10	13	2	36	84.45	15.55	15.82	1.95	24.07	45.80	12.36	Original.
Do	8	10	13	2	36	86.00	14.00	18.96	1.68	21.55	43.88	13.93	Silage.
Do	9	2	3	13	4 to 8	84.25	15.75	8.82	2.01	26.68	50.37	12.12	Original.
Do	9	2	3	13	4 to 8	86.35	13.65	11.56	1.32	23.99	48.73	14.40	Silage.
First flower in blossom	9	10	9	9	36	84.15	15.85	12.20	2.39	20.25	52.40	12.76	Original.
Do	9	10	9	9	36	85.00	15.00	15.93	3.13	18.32	48.47	14.15	Silage.
Do	8	0	1	8	4 to 8	79.75	20.25	7.13	2.13	28.89	51.16	10.69	Original.
Do	8	0	1	8	4 to 8	83.00	17.00	12.12	3.68	24.79	46.00	13.41	Silage.
Seeds not quite in dough stage	9	3	9	6	36	81.70	18.30	12.59	3.04	20.25	43.47	11.74	Original.
Do	9	3	9	6	36	83.50	16.50	14.62	1.65	27.57	42.46	13.70	Silage.
Do	7	7	2	3	4 to 8	79.00	21.00	10.25	2.61	24.95	50.59	11.69	Original.
Do	7	7	2	3	4 to 8	82.70	17.30	12.86	2.39	24.57	47.00	13.18	Silage.
First flower seeds well in dough stage.	9	0	10	1	36	79.85	20.15	10.25	2.18	20.66	57.05	9.86	Original.
Do	9	0	10	1	36	81.72	18.28	11.56	3.88	21.46	52.37	10.73	Silage.
Do	10	3	6	0	4 to 8	83.00	17.00	9.76	4.04	27.60	46.63	11.97	Original.
Do	10	3	6	0	4 to 8	84.00	15.90	11.06	5.14	22.10	48.75	12.95	Silage.
First flower seeds well beyond dough stage.	7	6	10	3	36	80.35	19.65	12.38	5.78	20.37	48.47	13.00	Original.
Do	7	6	10	3	36	80.60	19.40	13.63	5.35	23.45	42.49	15.08	Silage.
Do	8	10	6	0	4 to 8	81.80	18.20	11.75	1.68	24.84	49.83	11.90	Original.
Do	8	10	6	0	4 to 8	83.96	16.04	12.75	3.70	21.90	48.36	13.29	Silage.

DISCUSSION OF THE COMPOSITION OF SUNFLOWERS

In studying the results on the five stages of maturity, consideration must be given to the general trend of results rather than to slight differences which may occur at the different stages of growth, for the reason that it is extremely difficult to secure uniform samples. All percentages are based on the original weight of the plant, and were found by analysis of the anhydrous material.

DRY MATERIAL

In comparing the sunflower plants cut at different stages of maturity in the 4 to 8 inch spacing and the 36-inch spacing, there is seen very little difference in percentage of dry material, the 36-inch spacing showing only a slightly higher percentage of dry material over the 4 to 8 inch spacing. The plants in the 36-inch spacing show a more gradual increase in dry material throughout the five stages of maturity than the plants in the 4 to 8 inch spacing. The fifth stage of maturity of the sunflowers represents the stage of growth that was siloed in the large experiment station silos.

PROTEIN

Protein is consistently higher in all the stages of maturity when the plants were spaced 36 inches apart than when they were spaced from 4 to 8 inches. The first two stages of maturity of the 36-inch spacings were considerably higher in protein than the first two stages of the 4 to 8 inch spacings. In the last three stages, the advantage of high protein remains with the 36-inch spacings, but the differences in the percentage of protein in the two spacings were not so marked as they were in the first two stages.

CRUDE FIBER

Contrary to the general belief, it is seen that the crude fiber content of the sunflowers in both systems of plantings is not greater at the latter two stages than at the earlier stages of maturity. In fact, this table shows that the crude fiber is slightly less at the latter stage of maturity than at the earliest stage analyzed. The 36-inch spacings have less crude fiber than the 4 to 8 inch spacings. From the results it appears that the percentage of crude fiber does not increase until near the end of the growing period of the sunflower, when the seeds are matured and the stalk and leaves are dead. At this stage the percentage of moisture has decreased, the stalk has become hard and woody, and many leaves have fallen, thus increasing the crude fiber and materially lessening the feeding value of the sunflowers.

ETHER EXTRACT

No uniformity seems to exist in the relation of the ether extract in the two different systems of spacings at the different stages of maturity. In general, the 36-inch spaced plants show a larger percentage of ether extract than the 4 to 8 inch spaced plants. Sunflowers when planted closely together produce much smaller flowers, which do not always mature, hence the ether extract is less in amount. Plants spaced far apart not only produce larger flowers but in many cases more mature ones containing a considerable quantity of seed, which in turn contain a large amount of oil, thereby increasing the percentage of ether extract.

COMPOSITION OF LEAVES, STALKS, AND FLOWERS

The composition of leaves, stalks, and flowers was also determined. The results are given in Table II.

TABLE II.—*Composition of sunflower leaves, stalks, and flowers (1920)*

TABLE II.—*Composition of sunflower leaves, stalks, and stems (1920)*

Stage of growth.	Average height.	Average weight.	Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extract.	Crude fiber.	Nitrogen-free extract.	Ash.	Part of the plant analyzed.
	Ft. in.	*Lbs. oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Budding		2 8	36	77.20	22.80	26.20	2.72	8.33	42.85	22.80	Leaves.
Do		0 14	4 to 8	77.20	22.80	22.38	3.69	9.88	44.57	22.80	Do.
Do	8 3	8 4	36	85.60	14.40	8.56	1.38	27.38	52.13	10.55	Stalk and stems.
Do	9 8	3 ½	4 to 8	82.20	17.80	6.88	0.83	28.66	54.65	8.98	Do.
First flower in blossom		3 0	36	77.70	22.30	23.57	2.94	9.06	44.16	20.27	Leaves.
Do		1 1½	4 to 8	75.90	24.10	21.69	3.78	11.04	48.40	19.09	Do.
Do		0 15	36	81.20	18.80	16.69	4.71	11.16	58.67	8.77	Flowers.
Do		0 10½	4 to 8	80.80	19.20	15.81	4.57	4.87	66.50	8.25	Do.
Do	11 2	8 1	36	80.30	19.70	5.31	0.63	21.85	63.80	8.41	Stalk and stems.
Do	10 2	4 0	4 to 8	80.40	19.60	5.74	0.56	31.84	52.10	9.76	Do.
Seeds not quite in the dough stage		1 7	36	76.40	23.60	21.94	2.05	9.87	45.06	21.08	Leaves.
Do		1 5	4 to 8	78.20	21.80	24.19	2.97	10.91	41.71	20.22	Do.
Do		1 0	36	83.90	16.10	15.62	5.66	10.28	60.12	8.32	Flower.
Do		0 15	4 to 8	84.70	15.30	14.81	5.03	12.88	58.83	8.45	Do.
Do	8 4	5 0	36	81.00	19.00	5.31	0.51	27.98	57.17	9.03	Stalk and stems.
Do	10 9	4 10	4 to 8	84.20	15.80	6.12	0.49	36.94	46.37	10.08	Do.
First flower seeds well into the dough stage		0 15	36	76.80	23.20	20.80	3.44	8.98	46.75	20.03	Leaves.
Do		0 12	4 to 8	75.60	24.40	22.50	4.05	9.71	43.70	20.00	Do.
Do		1 14	36	84.00	16.00	15.56	9.48	20.40	46.56	8.00	Flower.
Do		0 14	4 to 8	82.00	18.00	13.87	5.38	11.92	61.23	7.60	Do.
Do	8 4	4 5	36	85.70	14.30	5.19	0.86	31.74	50.72	11.49	Stalk and stems.
Do	10 0	3 12½	4 to 8	80.40	19.60	5.25	1.76	32.58	52.30	8.11	Do.
First flower seeds well beyond the dough stage		2 8	36	77.30	22.70	24.25	4.05	8.78	42.88	20.04	Leaves.
Do		0 10½	4 to 8	75.00	25.00	20.19	4.16	11.06	42.43	22.16	Do.
Do		2 14	36	82.30	17.70	16.12	6.27	17.44	53.27	6.90	Flower.
Do		0 14½	4 to 8	82.80	17.20	14.31	5.94	15.60	56.28	7.87	Do.
Do	8 6	9 2	36	83.50	16.50	5.56	0.72	32.24	48.74	11.84	Stalk and stems.
Do	8 2	2 4	4 to 8	78.50	21.50	4.25	0.88	29.21	57.11	8.55	Do.

Table II is presented with a view of showing the distribution of the food nutrients in the leaves, flowers, and stalks. The table is largely self-explanatory, but a few significant facts will be discussed. The largest protein percentage content of the plant is found in the leaves. The flowers rank second and the stalks contain the smallest proportion.

When the different stages of maturity are compared in the two systems of spacing, it is seen that the percentage of protein in the leaves is quite constant throughout the various stages of growth. The 36-inch spaced plants showed a slightly higher percentage of protein in the leaves at the final stage of maturity as compared with the plants of 4 to 8-inch spacing. The percentage of protein found in the stalks and stems is the reverse of that found in the leaves when the two spacings are compared, while the percentage of protein in the flowers of both systems of plants is quite uniform. In the percentage of ether extract the flowers rank first, the leaves second, and the stalks the last in amount. The percentage of crude fiber is greatest in the stalks and stems and least in the leaves. The flowers contain an amount intermediate between the leaves and stalks, and as the flowers mature they show an increase in amount of crude fiber.

TABLE III.—*Percentage of leaves, stalks, and flowers (1920)*

Stage of growth.	Height.	Average weight of plants.	Distance apart in rows.	Flower.	Leaves.	Stalk and stems.
	Ft. in.	*Lbs. oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*
Budding	8 3	10 12	36	.....	23.1	76.9
Do	9 8	3 15	4 to 8	.....	22.2	77.8
1st flower in blossom	11 2	12 0	36	7.8	25.0	67.2
Do	10 2	5 12	4 to 8	11.4	18.0	70.6
Seeds not quite in the dough stage	8 4	7 7	36	13.5	19.3	67.2
Do	10 9	6 14	4 to 8	13.6	19.1	67.3
1st flower seeds well into dough stage	8 4	7 2	36	26.3	13.0	60.7
Do	10 0	5 1½	4 to 8	17.7	14.7	67.6
1st flower seeds well beyond the dough stage	8 6	14 8	36	19.0	17.2	63.8
Do	8 2	3 13	4 to 8	23.8	14.0	62.2

SUNFLOWER PLANT PERCENTAGE OF LEAVES, STALKS, AND FLOWERS

Table III is inserted to show the percentage of leaves, stalks, and flowers. Since the analyses of these different parts showed such widely different amounts of food nutrients, this table was introduced to show whether the two systems of plantings resulted in a different percentage of leaves, stalks, and flowers. A study of Table III indicates that under these two systems of plantings no consistent variations were noted in the ratios of leaves, stalks, and flowers. The percentage of leaves and stalks decreased throughout the growing period, while the percentage of flowers increased; this was true in both systems of plantings. The actual percentage of flowers, leaves, and stalks remain quite uniform throughout the various stages of maturity in sunflower plants which are grown at the two extreme distances in the row—namely, 4 to 8 and 36 inches.

TABLE IV.—*Acidity of sunflower silage (1920)*

Stage of growth.	Distance apart in rows.	Moisture.	Acids in 100 gm. silage juice.		Total acids.
			Nonvolatile acids.	Volatile acids.	
	Inches.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*
Budding	36	86.0	2.024	0.382	2.406
	4 to 8	86.35	2.616	.558	3.174
First flower in blossom	36	85.0	2.200	.566	2.766
Do	4 to 8	83.0	2.640	.653	3.293
Seeds not quite in dough stage	36	83.5	1.896	.509	2.405
Do	4 to 8	82.7	2.740	.600	3.340
First flower seeds well in dough stage	36	81.7	2.648	.728	3.376
Do	4 to 8	84.1	2.376	.613	2.989
First flower seeds well beyond dough stage	36	80.6	2.536	.700	3.236
Do	4 to 8	83.9	3.000	.623	3.623

Stage of growth.	Distance apart in rows.	Moisture.	Acids in 100 gm. wet silage.		Total acids.	Acids in 100 gm. anhydrous silage.		Total acids.
			Nonvolatile.	Volatile.		Nonvolatile.	Volatile.	
	Inches.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Budding	36	86.0	1.741	0.329	2.070	10.69	2.020	12.71
	4 to 8	86.35	2.260	.482	2.742	14.30	3.049	17.35
First flower in blossom	36	85.0	1.870	.481	2.351	10.68	2.747	13.43
Do	4 to 8	83.0	2.191	.542	2.733	10.70	2.646	13.35
Seeds not quite in dough stage	36	83.5	1.585	.425	2.010	8.09	2.170	10.26
Do	4 to 8	82.7	2.267	.496	2.763	10.84	2.371	13.21
First flower seeds well in dough stage	36	81.7	2.162	.595	2.757	9.71	2.657	12.37
Do	4 to 8	84.1	2.000	.516	2.516	10.59	2.730	13.32
First flower seeds well beyond dough stage	36	80.6	2.045	.564	2.609	8.53	2.350	10.88
Do	4 to 8	83.9	2.518	.523	3.041	13.10	2.723	15.82

ACIDITY OF SUNFLOWER SILAGE

Table IV shows that there is more acidity formed when sunflowers are siloed in the later stages of growth than when siloed in the earlier stage. This fact is different in corn silage, since immature corn always produces more acidity than corn siloed at a mature stage. The total amounts of acidity, however, found in these samples of sunflower silage easily fall within the amounts usually found in good sunflower silage. It must be remembered that kind of acids present is more of a criterion of good silage than quantity of acids. All silage samples made at the different stages of growth were classed as good samples when acidity, odor, and color were used as the criterion. The earlier stages would not be as suitable for silage purposes as the later stages, due to the large percentage of moisture which they contained. Even the last stage cut contained slightly higher than 80 per cent of moisture, but it must be remembered that these samples were not allowed to be exposed to the

sun after cutting, hence they represent the amount of moisture present immediately at the time of cutting. In the general farm practice of siloing sunflowers there is usually a loss of a few per cent of moisture by the slight wilting of the plants which takes place during the time of cutting and hauling to the silage cutter. The plants cut at this last stage of maturity were siloed in the large experiment station silos and produced excellent silage.

COMPOSITION OF SUNFLOWERS IN 1921

Additional work was carried on in 1921 on sunflowers planted singly and in hills at different distances apart in the row. The different distances apart in the rows selected were 8, 24, and 42 inches. In the hill system of planting there were one, two, three, and four stalks to the hill. All hills were planted 42 inches apart in the row. In both the drilled and hill systems the rows were 42 inches apart, this being the width of the planter.

The results of the analyses of the five stages of plants are given in Table V. All results except acre yield are given on the anhydrous basis.

In comparing the different systems of planting, 8, 24, and 42 inches apart in the row, and the hill systems, one, two, three, and four plants in the hill, it is noticeable that the 8-inch spacings produced the highest yield of all. This system is closely followed by the hill system of planting where three stalks were allowed to grow in each hill.

ANHYDROUS MATERIAL

The same conclusions as those found in 1920 are drawn when the amount of anhydrous material is considered. The 8-inch spacing is highest in amount with the three stalks in a hill system closely following. Very little distance is noted in any of the other systems of planting.

CRUDE PROTEIN

Crude protein gradually decreases as the sunflowers become more mature. The amount of protein is quite similar for all systems of plantings, averaging between 9 and 10 per cent in the final stages, the only exception to this being the four stalks in a hill system, which shows only 7.98 per cent crude protein in the final stage analyzed.

CRUDE FIBER

It is a noticeable fact that crude fiber increases up to the fourth stage, then decreases somewhat in the final stage examined. This fact holds true in both 1920 and 1921, with the exception of the 8-inch spacings grown in 1921.

ETHER EXTRACT

Ether extract is considerably higher in the last two than in the first three stages analyzed.

TABLE V.—*Composition of sunflowers (1921)*

Stage of growth.	Height.	Weight.	Distance apart in rows.	Acre tons.[a]	Number in hill.	Moisture.	Anhydrous material.	Crude protein.	Ether extract.	Crude fiber.	Nitrogen free extract.	Ash.
	Ft. in.	*Lbs. oz.*	*Inches.*			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Budding	6 6	1 5	8	.....	1	81.4	18.6	10.38	1.82	26.40	48.72	12.68
First flower in blossom	6 1	1 8½	8	.....	1	83.44	16.56	8.44	2.29	25.50	52.27	11.50
Seeds not quite in dough stage	6 1	1 14	8	.....	1	82.05	17.35	8.80	2.26	27.30	48.89	12.75
First flower seeds well into dough stage	6 1	2 1	8	.....	1	81.4	18.6	7.94	2.55	27.10	49.66	12.75
First flower seeds well beyond dough stage	6 0	1 15	8	11.36	1	77.4	22.6	9.32	10.02	28.15	41.76	10.75
Budding	6 0	2 6½	24	.....	1	82.4	17.6	11.60	1.72	26.10	46.53	14.05
First flower in blossom	6 0	2 13	24	.....	1	81.93	18.07	10.40	2.26	27.10	48.39	11.85
Seeds not quite in dough stage	6 4	3 8	24	.....	1	82.2	17.8	9.72	1.92	30.30	46.91	11.15
First flower seeds well into dough stage	6 6	4 5	24	.....	1	82.53	17.47	9.32	1.93	31.50	46.13	11.12
First flower seeds well beyond dough stage	6 0	4 14	24	7.72	1	79.4	20.6	10.90	7.38	29.10	41.83	10.79
Budding	5 10	5 3½	42	.....	1	84.14	15.86	13.90	1.63	26.20	43.30	14.97
First flower in blossom	5 5	5 2½	42	.....	1	85.53	14.47	12.80	1.70	27.50	44.63	13.37
Seeds not quite in dough stage	5 11	6 2	42	.....	1	83.57	16.43	11.35	1.76	25.90	49.39	11.60
First flower seeds well into dough stage	5 11	8 0	42	.....	1	83.8	16.2	11.98	2.25	31.70	42.40	11.67
First flower seeds well beyond dough stage	5 6	8 3	42	9.72	1	79.4	20.6	9.53	5.87	27.75	46.95	9.90
Budding	5 11	2 8½	42	.....	2	83.4	16.6	10.45	1.57	29.80	45.18	13.00
First flower in blossom	6 2	2 12	42	.....	2	83.67	16.33	10.65	1.97	27.80	46.11	13.47
Seeds not quite in dough stage	6 2	3 6½	42	.....	2	82.23	17.77	10.90	2.07	27.75	46.28	13.00
First flower seeds well into dough stage	5 10	4 5½	42	.....	2	82.07	17.93	10.71	3.75	30.15	43.24	12.15
First flower seeds well beyond dough stage	6 0	5 ½	42	10.28	2	79.2	20.8	9.75	5.56	27.46	47.98	9.25
Budding	6 4	1 15	42	.....	3	82.9	17.1	10.06	1.47	25.70	49.62	13.15
First flower in blossom	6 2	2 1	42	.....	3	81.87	18.13	9.35	2.11	25.70	51.39	11.45
Seeds not quite in dough stage	6 5	2 14	42	.....	3	82.43	17.57	10.00	1.75	31.30	44.30	12.65
First flower seeds well into dough stage	6 4	4 1	42	.....	3	83.0	17.0	10.50	2.43	30.40	45.02	11.65
First flower seeds well beyond dough stage	6 0	4 6	42	10.58	3	78.14	21.86	10.02	6.61	28.45	44.62	10.30
Budding	6 0	2 2	42	.....	4	84.54	15.46	10.25	1.32	23.80	52.38	12.35
First flower in blossom	6 1	2 ⅛	42	.....	4	82.4	17.6	10.30	1.90	27.04	49.01	11.75
Seeds not quite in dough stage	6 5	2 11⅓	42	.....	4	84.25	15.75	10.85	1.61	30.40	43.89	13.25
First flower seeds well into dough stage	5 10	2 12	42	.....	4	79.7	20.3	9.33	3.06	29.70	46.31	11.60
First flower seeds well beyond dough stage	6 3	2 11	42	9.58	4	78.6	21.4	7.98	6.32	26.10	48.70	10.90

[a] Acre yields were furnished by the agronomy department of the University of Idaho.

COMPARISON OF YIELDS, 1920 AND 1921

The yield per acre of sunflowers grown in 1921 was slightly less than half the yield secured in 1920, due to dry weather. The total precipitation for each month during the growing season is given in the following table:

TABLE VI.—*Precipitation during the growing season*

Month.	1920.	1921.	Month.	1920.	1921.
	Inches.	*Inches.*		*Inches.*	*Inches.*
April	2.72	2.86	July	0.54	0.19
May	1.35	1.80	August	1.22	0.30
June	1.16	1.47	September	2.52	1.43

CONCLUSIONS

A comparison of the results on sunflowers grown in Idaho and those grown by Shaw and Walters at Beltsville, Md., shows some striking facts. Probably, due to climatic conditions, Shaw and Wright were able to secure a more advanced stage of maturity of their sunflower crop as the last stage represented the production of good hard seed. As has been stated before, it is seldom possible to secure such mature sunflowers in certain portions of the Northwest. The crop grown for this experimental work is a good example, since the last stage analyzed represents the maximum growth. At this period a frost occurred and the sunflowers were siloed.

In content of dry material Idaho sunflowers when siloed represent Shaw and Wright sunflowers matured in stages between rays partly fallen and rays all fallen. It appears from these results, and results of previous years, that the crude protein is considerably higher in sunflowers grown in the Palouse country of Idaho than in Maryland, one crop grown in 1920 representing 12.38 per cent, and one in 1921 10 per cent, as against approximately 7 per cent in Maryland for the same stage of maturity. The sunflowers grown in Montana, analyzed by Blish, show lower percentages of protein than sunflowers grown in the Palouse section of Idaho. From these results it would appear unfair to compare sunflowers grown in different parts of the United States for relative food value, since the composition of sunflowers may vary widely, owing to the differences in climatic and soil conditions.

The 1920 results on the composition of sunflowers at different periods of growth show that the crude fiber does not continue to increase as the plant passes through the various stages of maturity. Similar results were obtained with the 1921 crop. The percentage of crude fiber found in the sunflowers cut at the last stage of growth was slightly less than in the more immature stages studied. From these data, it appears that there is practically no gain in crude fiber during the growth of the sunflower plant throughout the five stages of maturity included in this investigation. The crude fiber may increase materially if the sunflowers are allowed to mature completely. At this time the leaves are dead and partly fallen and the stalk becomes hard and woody. Silage made from sunflowers in this stage of maturity is high in crude fiber and is not palatable. It is possible that some of the unfavorable results which have been noted in isolated cases with sunflowers have been due to the fact that sunflowers were allowed to mature too far before they were cut for silage.

TIME FOR CUTTING

In considering the proper stage for cutting sunflowers in Idaho, either of the last two stages could be profitably used for silage, as there is little material difference in the analyses for the two stages in either the 1920 or 1921 crops. In some sections where sunflowers mature slightly more than in the Palouse country of Idaho, it might be profitable to cut them a little later than those of the last stage included in this investigation. In any event, cutting should not be delayed much beyond the last stage studied, for then the stalks become hard and woody and a silage is obtained which is unpalatable and not relished by the stock.

The moisture content of the sunflower plant grown in the last two stages is approximately 80 per cent. This percentage of moisture is slightly higher than is usually desired in a crop intended for the silo. When the green material contains too much moisture, there is danger of a loss of considerable juice, which means a loss of soluble food nutrients. In crops containing a high sugar content, a high moisture content means also a high acid content. Shaw and Wright state that "a high moisture in the plant is usually associated with high acid silage." This holds true for corn silage, but an inspection of Table I shows that it does not always hold true for sunflowers. In the case of sunflowers a high moisture content results chiefly in a loss of soluble food nutrients in the juice.

If the sunflowers seem to have too high a moisture content when cut, this can be materially decreased by allowing the plant to wilt for a short period. Blish, of Montana, has shown that excellent silage resulted when sunflowers were cut and allowed to wilt before siloing until the moisture content was 72.24 per cent. A moisture content for sunflower silage in Idaho can vary from 70 to 80 per cent, the writers recommending approximately 75 per cent as an average.

DISTANCE APART IN ROW

When the two different distances of planting in the row are considered for 1920, it appears that the single plants growing 36 inches apart in the row rank slightly higher in food nutrients than the sunflowers planted every 4 to 8 inches. The analyses of the sunflowers, and the sunflower silage, both show a slight advantage over the closer plantings. The yield must be considered in deciding which system of planting is most profitable. The yields for the two spacings determined by the agronomy department showed 22.32 tons for the 36-inch spacings and 20.23 tons for the 4-inch to 8-inch spacings. However, because of the difference in the contour of the land upon which the sunflowers were grown, these yields can not be considered as final.

The yields of sunflowers grown in 1921 show results which are contrary to the findings of 1920. The 8-inch spacings gave a higher yield than the 42-inch spacings. Under the conditions that existed in 1921, very little difference was noted in the yields of the sunflowers drilled 8 inches apart and the sunflowers planted in hills containing two or three stalks. Four stalks in the hill did not yield as well as the hills containing two and three stalks.

From the data presented on the two years of sunflowers it is apparent that the different climatic conditions, chiefly the proper distribution of moisture throughout the life cycle of the sunflower plant, affect the yield of sunflowers when planted at different spacings in the row. The results vary from year to year in the Palouse country, because of variable

climatic conditions, to such an extent that no specific recommendations can be made as to the best systems of planting. Where irrigation is practiced, and the moisture supply controlled, more regular yields would be expected under the different systems of planting. It is hoped that these studies on sunflowers grown under irrigation will be continued. The results in this paper are representative of what may be expected when sunflowers are grown in sections of the United States where climatic conditions and altitude are similar to those in the Palouse country. The choice of the system of planting sunflowers, grown without irrigation, must necessarily rest with the farmer, depending upon whether it is more feasible to harvest and silo the large type of sunflowers rather than the smaller type. In choosing the system of planting, the farmer should consider the difference in labor involved in harvesting the large or small type of sunflowers.

EFFECT OF DIFFERENT CONCENTRATIONS OF MANGANESE SULPHATE ON THE GROWTH OF PLANTS IN ACID AND NEUTRAL SOILS AND THE NECESSITY OF MANGANESE AS A PLANT NUTRIENT[1]

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INTRODUCTION

In a study of the literature on the nutrition of plants, one is impressed with the lack of definite knowledge concerning the necessity of a number of elements frequently found in appreciable amounts in the ash of different species.

For many years it has been taught and accepted by agronomists and plant physiologists that only 10 of the elements are essential for the normal growth and maturation of plants. These essential elements are carbon, hydrogen, oxygen, nitrogen, calcium, magnesium, potassium, phosphorus, sulphur and iron. In addition to these, it is also well known that as many as 20 or 30 others are frequently found in small amounts in plants which have grown under natural conditions.

Within recent years many data have been obtained which indicate that a few of these so-called nonessential elements may have important functions in the plant's economy. During the past 25 years, perhaps, more attention has been given to the acquisition of knowledge concerning the occurrence, distribution, and probable functions of manganese in soils and plants than to any other one of the elements referred to.

The number of contributions to the literature on the relation of manganese to various phases of agriculture number no less than 100. As a matter of convenience these contributions may be divided into two general classes, depending upon the object to be attained. Investigators of one class, apparently, have been interested in determining whether or not manganese has any commercial value from the standpoint of a necessary fertilizer, while those of the other class have sought to determine whether or not manganese is an essential element in the vegetable economy and, if so, to assertain its functions.

HISTORICAL

Manganese was discovered by Scheele (*9*)[2] in 1774. He found that the soil contained small amounts of this element and that it was assimilated by plants that grew in the soil; however, he made no effort to determine whether manganese was necessary for plant growth.

In 1864 Sachs (*8*) conducted experiments, the purpose of which was to determine whether or not manganese was necessary in plant economy. He proved that manganese could not replace iron in plant growth, but

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This investigation, begun at the Kentucky Agricultural Experiment Station in 1916, was continued, with the consent of the Director, at Cornell University where part of the results were offered in partial fulfillment of the requirements for the degree of doctor of philosophy. Since September, 1922, the work has been carried on at the Kentucky Station. The paper presents in part the results of the investigation at Cornell, the earlier results, and the recent findings at the Kentucky Station.

[2] Reference is made by number (italic) to "Literature cited," p. 793–794.

he did not prove that manganese was also necessary when iron was available.

In 1883 Yoshida (*12*) isolated from the sap of the lac tree a product possessing peculiar enzymic properties which he was unable to explain.

In 1894 Bertrand (*1*) undertook further work on the nature and composition of the product which had been isolated from the sap of the lac tree, and found that it contained considerable manganese, which gave to the product its peculiar enzymic properties. After several years of research on the relation of manganese to the growth of plants he concluded that this element is necessary for the normal growth of both autotrophic and heterotrophic plants and that its functions can not be performed by any other element.

In 1914 Brenchley (*2*) published the results of an investigation concerning the effect of manganese sulphate on the growth of barley seedlings. She found that with a concentration of 1/100,000 of $MnSO_4$ and less a decided stimulation in the growth was produced; with greater concentrations than this, however, toxic effect resulted. In her conclusion with respect to the function of manganese in plants she suggests the probability that manganese may prove to be an essential element in the economy of plant life, even though the quantity usually found in plants is very small.

Kelly (*5*) made a study of the effect produced on different species of plants when grown in a natural soil containing a large amount of manganese as compared with the effect produced on the same species when grown on a soil containing only a normal amount of manganese. From his results he concluded that the small amount of manganese occurring in soils probably performs a twofold function in plant growth: (1) It acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of calcium and magnesium, perhaps by partially replacing calcium from insoluble combinations, but especially through a direct effect by which the absorption of calcium is increased and that of magnesium is decreased.

McHargue (*6*) grew wheat plants to maturity in solution cultures free from and containing manganese and found that in the absence of manganese the plants became etiolated and did not develop in a normal way, thus showing that manganese is essential to the normal growth and maturation of this plant.

Previous to 1902 but little attention had been given to the occurrence and distribution of manganese in soils. During that year Ewell (*3*) made an interesting observation with respect to the occurrence of soluble salts of manganese in a certain small area of soil which failed to produce crops in a normal way. The results of his investigation showed that the small nonfertile area of soil contained relatively large amounts of soluble salts of manganese, while the adjacent fertile soil contained none. He assumed that the difference in the fertility of the two soils was due to the presence of the soluble compounds of manganese. The publication of his conclusions apparently awakened a wide interest regarding the rôle manganese plays in soils.

In 1907 the United States Bureau of Soils (*10*) inaugurated a series of experiments with manganese at the Arlington Experiment Farm. Manganese sulphate was applied at the rate of 50 pounds per acre to an acid silty clay loam soil, and crops were grown on the treated and untreated soil for six years. The application of manganese to the acid soil resulted

in decreased yields; however, upon neutralizing the acidity in the soil by the addition of calcium carbonate the plots receiving manganese produced very marked increases in yields, thus showing that the reaction of the soil is an important factor in determining whether or not an application of manganese sulphate will exert a beneficial effect on plant growth.

Funchess (*4*) has obtained data which tend to show that some acid soils contain soluble salts of manganese. He also offers the suggestion that the toxic effect associated with soil acidity may be due in part to soluble salts of this element. He describes experiments with acid soils that contained soluble salts of manganese and were toxic to the growth of plants. He found that the toxic effect of the soil was destroyed after neutralization with calcium hydroxid.

In 1920 Olaru (*7*) published results showing the effect of manganese on some of the more important microorganisms concerned with nitrogen transformations in soils and leguminous plants. With pure cultures of *Bacillus radiocicola*, *Azotobacter chroococcum*, *Clostridium pasteurianum*, and *Micrococcus ureae* and with concentrations of manganese sulphate varying from zero to 10 mgm. per liter he obtained a marked stimulation in the functions of each of these organisms. The maximum stimulation appears to have been obtained with concentrations of about 1 mgm. of manganese per liter. He therefore concludes that the small amount of manganese occurring in the soil serves a very useful function with respect to the organisms concerned in nitrogen transformation.

Robinson (*11*) has determined the manganese content of 26 different and representative soil types in this country. The maximum amount found in any soil was 0.51 per cent, the minimum 0.01 per cent, and the average 0.20 per cent of MnO, respectively. Various other reports concerning the amount of manganese contained in the soils of this and other countries will average approximately 0.10 per cent of this element, which is as much as the phosphorus and sulphur contained in soils that produce average yields of field crops.

In the foregoing review it has been the aim of the author to refer to those contributions on the subject which contain the more modern views concerning the probable function of manganese in its relation to agriculture.

The purpose of this investigation was, first, to show the effect of increased concentrations of manganese sulphate on the growth of plants in certain soils, and, secondly, to determine definitely if manganese is an essential element in the plant economy.

EXPERIMENTAL DATA

Citation (*4*) is a contribution whose data indicate that soluble salts of manganese are associated with and may in part be responsible for the toxicity of acid soils. To obtain further data on this subject, a series of experiments was planned in which plants were grown in acid and in neutralized portions of the same soil to which were added equal and graduated amounts of manganese sulphate. The soils selected for the experiments were not only acid but were lacking in capacity to produce good crops without the addition of certain plant nutrients.

EXPERIMENTS WITH VOLUSIA SILT LOAM SOIL

Volusia silt loam soil has a brownish-gray color, is low in organic matter, and contains considerable clay. It requires about 2,000 pounds of calcium carbonate per acre foot to neutralize its acidity.

About 500 pounds of this soil were obtained in a pasture field on Turkey Hill, Tompkins County, N. Y. The soil was well mixed and allowed to air-dry. Representative samples were taken for determination of the total and water-soluble manganese.

The total manganese content was determined by the potassium bisulphate fusion and the colorimetric periodate method. The amount of manganese found by this method was 0.08 per cent, or 800 parts per million of the air-dry soil. The amount of manganese dissolved by digestion with distilled water was 6.25 parts per million of the air-dry soil.

For pot experiments, 4,000 gm. portions of the air-dry soil were weighed into clean 1-gallon earthenware jars without drainage. Two series of 12 jars each were prepared, one with and the other without calcium carbonate, as shown in Table I, each treatment being made in duplicate. The mineral nutrients consisted of 10 gm. calcium nitrate, 10 gm. dipotassium phosphate, and 5 gm. magnesium sulphate per jar.

TABLE 1.—*Pot treatments in Series I and II*

Pot No.	Treatment, Series I.	Treatment, Series II.
1 and 1a...	No treatment.....	20 gm. $CaCO_3$.
2 and 2a...	Mineral nutrients only.....	Mineral nutrients and 20 gm. $CaCO_3$.
3 and 3a...	Mineral nutrients and 5 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 5 parts per million Mn.
4 and 4a...	Mineral nutrients and 10 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 10 parts per million Mn.
5 and 5a...	Mineral nutrients and 50 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 50 parts per million Mn.
6 and 6a...	Mineral nutrients and 100 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 100 parts per million Mn.

Purple-top radish seeds were sown in each pot and the moisture content of the soil in each pot was brought to one-half saturation, by weight, with distilled water. After the seedlings were up they were thinned to 10 plants in each pot and the moisture content kept at approximately one-half saturation with distilled water during the time the plants were making their growth.

Immediately after the crop of radishes was harvested soybeans were planted in each of the pots without further treatment of the soil. After the beans had reached the proper size they were thinned to 6 plants in each pot and allowed to grow until they were approaching maturity, the moisture content being meanwhile kept up to about one-half saturation with distilled water.

Plate 1, A, represents five pots from series No. 1. Pot No. 1 is one of the control pots and represents the productivity of the soil without treatment. Pot No. 2 shows the effect of the addition of appropriate amounts of phosphorus, potassium, calcium, magnesium, nitrogen and sulphur to the soil. This is one of the pots that received mineral nutrients

only and no manganese. Pot No. 3 received 5 parts per million of manganese in addition to the mineral nutrients. Pot No. 4 received 10 parts per million of manganese. Pot No. 5 received 50 parts per million of manganese. It is therefore readily apparent that 50 parts per million of manganese in the form of manganese sulphate are sufficient to produce toxicity in this soil. None of the seeds came up in the soil receiving this and greater concentrations of manganese sulphate. It is quite evident from Plate 1, A, that the maximum tolerance for manganese sulphate in this soil and with this particular plant lies between 10 and 50 parts per million.

Table II shows that the plants in the corresponding duplicate pots which are not shown made a similar growth.

There is no noticeable difference in the growth of the plants receiving the 5 and 10 parts per million of manganese, as compared with those that received no additional manganese. It is apparent that the small amount of manganese contained in the soil was sufficient for the requirements of the plants, under the conditions described.

The growth attained by the plants in the neutralized Volusia soil is shown in Plate 1, B, in which pot No. 1 is one of the control pots to which calcium carbonate was added. Comparison with the corresponding pot in Plate 1, A, shows that the plants made a slightly better growth in the neutralized soil than in the acid soil. Pot No. 2 received mineral nutrients and calcium carbonate and the plants did not make as good growth as did those in the corresponding pots with the acid soil. Pot No. 4 is one of two to which 10 parts per million of manganese was added. Neither of the two pots of soil that received 5 parts per million of manganese is shown in the photograph, but it will be seen in Table II that the plants that received 5 parts per million of manganese in series No. 2 made a slightly better growth than did the plants in the corresponding pots of soil in series No. 1. It will also be observed in Table II that the plants in the pots of soil that received 10 parts per million of manganese made a decidedly better growth in the neutralized soil than did the plants in the corresponding pots in the acid soil. Pot No. 5 shows the growth the plants attained in the neutralized soil to which 50 parts per million of manganese was added. It will be recalled that in the corresponding pots in the acid soil, the seeds did not germinate because of the toxic effect produced by an excess of manganese, while with the same concentration of manganese in the neutral soil the plants made a fair growth, though apparently retarded by an excess of this element. This experiment illustrates in a very striking way the effect of calcium carbonate in reducing the toxicity produced by an excess of manganese sulphate. In pot No. 6, to which was added 100 parts per million of manganese, no plants grew.

In Table II it will be observed that the applications of manganese to the acid soil reduced the yields, as shown by the green and dry weights of the plants, whereas with the same concentrations of manganese in the neutralized soil there was an appreciable gain.

TABLE II.—*Weights of radish and soybean plants grown in Volusia and Dunkirk soils, with and without nutrients, $MnSO_4$ and $CaCO_3$, duplicate pots*

		Acid reaction of soil.				Neutral reaction of soil.				
Crop.	Soil.	No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Green weight of radish tops and roots.	Volusia....	45.0	212.5	167.5	152.5	52.5	151.0	178.5	194.0	36.5
	Dunkirk..	92.5	240.5	205.0	101.0	95.0	129.0	185.0	222.5	146.5
Dry weight of radish tops and roots.	Volusia....	3.3	12.4	10.0	9.7	3.4	10.7	12.9	13.4	4.2
	Dunkirk..	7.3	15.8	13.2	8.8	7.8	7.7	12.3	13.8	10.1
Green weight of soybeans.	Volusia....	38.8	65.5	50.0	28.0	42.0	42.0	60.5	45.5	11.8
	Dunkirk..	30.3	67.8	41.3	20.5	27.5	27.5	50.8	44.3	48.5
Dry weight of soybeans.	Volusia....	4.4	12.5	9.6	6.0	9.3	9.9	12.5	10.1	2.6
	Dunkirk..	6.9	11.9	8.2	3.6	6.2	7.1	9.5	8.8	10.0

EXPERIMENTS WITH DUNKIRK CLAY LOAM SOIL

Two series of experiments similar to those just described with the Volusia soil were conducted at the same time and under the same conditions with the Dunkirk clay loam soil. Apparently this soil is slightly more productive than the Volusia silt loam soil. It has an acid reaction and responds to applications of certain plant nutrients.

A manganese determination on the Dunkirk soil gave 0.093 per cent of this element. The amount of water-soluble manganese found was 9.30 parts per million of the soil.

The radish plants which were grown on the Dunkirk soil under the same conditions as those previously described on the Volusia soil were harvested on the same day and treated in like manner for the green and dry weights, which are given in Table II.

No photographs were made of the plants grown in the Dunkirk soil; however, it will be seen from the green and dry weights of the plants, shown in Table II, that results somewhat comparable to those obtained with the Volusia soil were obtained under similar treatments with this soil. In series No. 1 the maximum yields occurred with the applications of mineral nutrients. Addition of manganese gave a diminution in yields as compared with the pots receiving mineral nutrients alone.

In series No. 2, application of calcium carbonate to the soil of pots No. 1 and 2 affected the growth of the plants but little as compared with the corresponding pots in series No. 1, in which the plants grew in acid soil. It thus appears that the application of calcium carbonate under these conditions had no effect as far as the growth of the plants was concerned. There is an increase which is apparently due to the applications of manganese in this series, as compared with the pots receiving mineral nutrients alone. This is in accord with the results obtained with applications of manganese to the neutralized Volusia soil.

Equal quantities of the radish tops grown in duplicate pots were combined to make a composite sample for chemical analysis, the results of which are given in Table III.

TABLE III.—*Analyses of composite samples of radish tops grown in Volusia and Dunkirk soils, with and without the addition of nutrients, manganese sulphate, and calcium carbonate (results calculated on moisture-free basis)*

		Acid reaction of soil.				Neutral reaction of soil.				
Element.	Soil.	No treatment.	Minerals	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ash	Volusia	21.89	25.60	25.51	24.45	22.06	24.96	25.49	26.00	21.71
	Dunkirk	20.15	24.83	24.58	26.76	17.55	19.06	25.08	25.56	25.45
Fe	Volusia	.077	.059	.050	.091	.047	.095	.096	.071	.08
	Dunkirk	.073	.073	.073	.073	.074	.084	.095	.091	.11
Mn	Volusia	.046	.035	.058	.94	.054	.046	.070	.087	1.15
	Dunkirk	.024	.024	.056	.67	.019	.021	.034	.059	.84
Ca	Volusia	3.86	3.59	3.12	3.25	3.01	4.39	4.44	4.54	2.81
	Dunkirk	3.10	3.32	3.23	3.95	2.72	2.85	2.91	2.96	2.98
Mg	Volusia	.46	.45	.41	.38	.43	.38	.40	.44	.38
	Dunkirk	.43	.40	.42	.43	.45	.42	.41	.42	.34
P	Volusia	.25	.25	.78	.72	.24	.70	.70	.65	.68
	Dunkirk	.27	.56	.55	.50	.25	.59	.58	.61	.60
K	Volusia	4.56	4.72	6.67	7.47	6.94	4.33	5.55	6.52	6.35
	Dunkirk	4.56	4.56	7.69	7.99	7.31	3.52	3.16	7.83	7.24
N	Volusia	6.04	6.79	6.58	6.71	6.15	6.48	6.52	6.45	7.81
	Dunkirk	5.56	6.77	7.22	6.51	3.19	3.58	5.17	5.04	5.56

An examination of the results shown in Table III reveals some points of interest. The plants which grew in the untreated soil contained less mineral matter than did those to which mineral nutrients were added. The addition of manganese to the soil in series No. 1 did not increase the mineral content of the plants receiving it, whereas it appears to have done so in the neutralized soil, except in pots 9 and 10, in which toxicity was produced. About as much mineral matter was taken up by the plants grown in the soil to which calcium carbonate was added as where it was omitted in the corresponding control pots of soil in series No. 1. A little more calcium was taken up by the plants under neutral conditions of the Volusia soil than under acid conditions; however, a little less calcium was found in the plants that grew in the neutralized Dunkirk soil than in those that grew in the acid soil. A larger percentage of potassium was found in the plants that grew in the acid Volusia and Dunkirk soils to which manganese was added than in those plants that grew in the soil to which no manganese was added. There was an increase in the percentage of nitrogen in the plants to which manganese was added in the neutral Dunkirk soil, while the percentage of nitrogen was nearly constant in the other experiments.

It appears, therefore, that in the soils dealt with in the foregoing series of experiments with radish plants, there has been a slight gain in each of the soils where manganese was added to the neutral soil in connection with mineral nutrients. Under acid conditions there has been a diminution in the yield of the plants receiving manganese when compared with the pots receiving mineral nutrients alone. All the soils have responded vigorously to an application of mineral nutrients when compared with no treatment.

An inspection of the green and dry weights of the soybean plants in Table II indicates that a more favorable growth was obtained in the neutralized soil plus manganese than in the acid soil to which manganese

was added. The protective action of calcium carbonate against the toxic properties of an excess of manganese sulphate is demonstrated with soybeans. The plants receiving 50 parts per million of manganese in the neutral soil made a moderate growth, while in the corresponding pots of acid soil no plants were produced.

After the dry weights were determined the leaves and stems of the soybean plants in duplicate pots were combined to make a composite sample for chemical analysis. The results of the analyses are given in Table VI.

TABLE IV.—*Analyses of composite samples of soybean plants grown after radish plants in Volusia and Dunkirk soils, with and without the addition of nutrients, $MnSO_4$ and $CaCO_3$ (results calculated on moisture-free basis)*

		Acid reaction of soil.				Neutral reaction of soil.				
Crop.	Soil.	No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Ash	Volusia	8.38	12.43	13.86	14.24	9.48	12.52	12.59	13.15	10.72
	Dunkirk	9.58	13.17	13.98	14.12	11.36	12.50	13.19	13.86	9.37
Fe	Volusia	.059	.078	.078	.069	.068	.059	.071	1.06	.114
	Dunkirk	.070	.070	.066	.051	.050	.052	.047	.052	.082
Mn	Volusia	.012	.012	.186	.303	.012	.014	.027	.042	.204
	Dunkirk	.013	.019	.162	.277	.069	.105	.346	.697	.186
Ca	Volusia	1.19	1.69	1.89	1.81	2.05	2.05	2.04	1.96	1.62
	Dunkirk	1.90	1.87	1.60	2.12	2.08	1.62	1.88	1.84	1.71
Mg	Volusia	.31	.29	.30	.28	.32	.30	.27	.28	.24
	Dunkirk	.33	.36	.31	.29	.31	.34	.28	.25	.36
P	Volusia	.13	.31	.35	.43	.18	.27	.24	.22	.36
	Dunkirk	.23	.45	.50	.55	.27	.48	.46	.40	.41
K	Volusia	1.43	3.40	3.62	3.92	1.40	3.12	3.03	3.05	3.03
	Dunkirk	1.34	2.80	3.39	2.20	3.51	3.71	3.51	3.34	3.21
N	Volusia	3.40	4.06	4.07	4.12	3.69	4.16	3.91	4.00	4.49
	Dunkirk	1.62	4.25	3.85	3.76	1.86	3.82	3.80	3.76	3.96

Table IV shows that the smallest percentage of ash was taken up by the plants grown in the untreated soils, while the largest percentage was found in the plants that had grown in the acid soil and to which 10 parts per million of manganese was added. Considerably more manganese than iron was taken up by the plants that grew in each of the acid soils to which manganese was added. Under neutral conditions more manganese than iron was absorbed by the plants that grew in the Dunkirk soil. The results for calcium vary to some extent between the two soils but apparently more calcium was taken up by the plants that grew in the neutralized soil. The plants that grew in the Dunkirk soil contained more phosphorus than those that grew in the Volusia soil. The amounts absorbed under acid and neutral conditions were nearly equal in the Dunkirk soil. Considerably more potassium was taken up by the plants receiving mineral nutrients and manganese than by either the untreated or the soils to which only calcium carbonate was added.

In the foregoing experiments with soils, the purpose was to determine the effect produced on the growth of plants in an acid and a neutral soil to which was added graduated amounts of manganese sulphate. It is readily apparent, therefore, that while such methods of experimentation are very important from an economic standpoint, they do not afford the desired proof to determine whether or not manganese is essential in the plant's economy.

The preparation of a medium that will, when the test is applied, show no manganese, requires certain procedures and precautions which, in so far as the writer has been able to discover, have not previously been taken into consideration in experiments planned to show the effect the absence of this element may have on the growth of plants. Tests for manganese in many different samples of chemical reagents which are used for plant nutrients show that manganese-free chemicals are rarely if ever found. So intimate is the association of manganese with iron that a manganese-free iron compound could not be purchased from dealers in chemicals and it was necessary to prepare it in the laboratory. Therefore in order to eliminate all possible sources of contamination with manganese it is necessary to test and purify all the reagents that enter the nutrient medium. Even though manganese has been eliminated from the nutrient medium it is necessary to grow plants until they approach maturity before any definite conclusions can be reached with respect to the effect of this element on their growth. The seeds of those plants with which the writer has carried on experiments apparently contained enough manganese to maintain a normal growth for the first six or eight weeks.

After having procured reagents that were proved by chemical tests to be free from manganese, a Knop's nutrient solution was made for water culture experiments.

Alaska garden pea seeds were germinated on moist cheesecloth in a porcelain-lined pan. When the seedlings were of the proper size they were transferred and held in place by means of plugs of cotton in holes made in squares cut from boards which were made to fit over the mouths of well-glazed one-half gallon earthenware jars. An attempt was made to waterproof the boards by keeping them submerged in paraffin kept near the boiling point for several hours. Although the squares of wood afford excellent means of support for the plants, they proved to be unsatisfactory in these and in previous experiments from the fact that the lower surface of the board, which is close to the top of the nutrient solution, affords favorable conditions for the growth of molds which, unless daily attention is given to washing them away, very soon attack the roots of the plants.

More recently supports have been made of well-glazed earthenware and no trouble from molds has been experienced.

The earthenware jars were filled nearly to the mouth with the Knop's solution and when the covers containing the seedlings were on, the roots were submerged in the solution. The pots containing the plants were kept at a suitable temperature for the growth of the peas in the greenhouse and the nutrient solutions were changed twice each week. The water lost by transpiration in the meantime was replaced with distilled water, thus keeping the concentration approximately constant.

Previous to starting the experiment new pots were obtained and tested for soluble manganese in the following manner: Three of the new pots were taken at random and filled to near the mouth with a mixture of equal parts of 1:1 nitric and hydrochloric acids and placed over holes on a water bath and kept at the temperature of boiling water for 48 hours. The mouths of the pots were covered with watchglasses during the digestion. The solutions were then transferred to porcelain dishes and brought to dryness. The residues were tested for the presence of manganese, and only a slight trace was found from any of the pots. The writer considers this a negligible source of manganese as it was thought

that even the trace found in the acid extraction would not be available to the plants growing in a Knop's solution.

Twelve Alaska pea seedlings were placed in each of four jars containing a Knop's solution. At the beginning of the experiment 1 part of manganese in the form of the sulphate to 1,000,000 parts of the solution was added to each of two of the pots. After the plants had been growing three or four weeks the concentration of manganese was increased to five parts per million of the Knop's solution. One cubic centimeter of a manganese-free ferric chlorid solution which contained 0.001 gm. iron was added to each of the pots at the time the solutions were changed.

During the first four or six weeks of growth there was little or no difference in the size or appearance of the plants. Therefore the plants which received no manganese except that contained in the seed began to show effects which can not be accounted for in any other way except for a lack of this element. The first effect to be observed was that the young buds as they unfolded were yellowish instead of a normal green color and later became flecked with small specks of brown, which was not observed at any time on the plants receiving manganese. The etiolated condition became more pronounced as the time progressed and finally resulted in the top branches dying back. The green and dry weights of the 12 plants that grew in each of the 4 pots were determined.

The average dry weights of the pea plants show an increase of 49.47 per cent in the total dry matter produced in the plants which were grown in the presence of manganese when compared with those plants that grew in a Knop's solution to which no manganese was added. There was also an increase of 24.63 per cent in the mineral nutrients assimilated, exclusive of nitrogen, in the plants that grew in the Knop's solution containing manganese. The plants which did not get any manganese contained a little more nitrogen than those to which manganese was added. The plants from which manganese was withheld contained only traces of this element, which was no more than could have been derived from the seeds from which the plants grew. It therefore appears that the trace of manganese derived from the seeds of the plants was not sufficient to maintain a normal metabolic process during the latter part of the time the plants were growing.

The duplicate sets of plants were combined to make a composite sample for chemical analysis and the results are given in Table V.

TABLE V.—*Analyses of pea, soybean, corn, and cowpea plants grown in media free from, and containing manganese (results expressed of the moisture-free material)*

	Ash (crude).	Iron (Fe).	Manganese (Mn).	Calcium (Ca).	Magnesium (Mg).	Potassium (K).	Phosphorus (P).	Nitrogen (N).
Alaska pea plants:	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Manganese	12.85	0.041	0.179	1.85	0.50	2.05	0.58	2.97
No manganese	15.41	.040	Trace.	2.41	.98	2.71	.68	4.71
Soybean leaves:								
Manganese	15.00	.051	.032	.80	.56	1.58	.62	3.42
No manganese	15.99	.094	Trace.	.82	.62	1.11	.48	3.85
Soybean stalks:								
Manganese	13.07	.036	.010	.57	.37	1.08	.45	1.69
No manganese	16.67	.033	None.	.68	.39	1.35	.46	2.21
Cowpea plants:								
Manganese	17.24	.046	.036	1.70	.54	5.56	.72	2.57
No manganese	20.14	.065	.003	1.32	.61	7.88	.51	4.51
Corn leaves:								
Manganese	8.71	.029	.040	.23	.31	3.80	.40	.22
No manganese	10.28	.034	.001	.24	.36	4.07	.35	1.47
Corn stalks:								
Manganese	6.37	.014	.003	.08	.13	3.02	.42	.77
No manganese	8.24	.011	Trace.	.10	.13	3.52	.37	.95

SAND CULTURE EXPERIMENTS

A quantity of quartz sand of medium sized grains was obtained for sand cultures. The sand, from external appearance, was good quality glass sand. Upon digesting 1,000 gm. in a mixture of 1:1 hydrochloric and nitric acids on a hot water bath for several hours, filtering out and washing the residue free of acids, it was found upon testing portions of the filtrate that the sand had contained small amounts of iron, manganese, zinc, arsenic, calcium, and magnesium. The residue of sand was snow white, except for a few black particles, which resisted further treatments of strong acids to dissolve them. A small amount of the black particles were separated, ground in an agate mortar, and fused with potassium bisulphate. The fused mass was extracted with dilute sulphuric acid and tested for manganese, but none was found.

Several hundred pounds of the sand were then washed with a mixture of nitric and hydrochloric acids and afterwards with distilled water until free of chlorids, as shown by a test with silver nitrate. The sand was then transferred to large, shallow, porcelain-lined agateware pans and dried. After the sand was dry 4,000 gm. portions were weighed into clean 1-gallon earthenware pots.

The sand prepared in this way possessed rather strong absorptive powers when wet with a Knop's nutrient solution. It, therefore, was necessary to saturate the absorptive capacity of the sand with respect to the plant nutrients before plants could be grown. This was accomplished by adding the following amounts of manganese-free mineral nutrients to each 1 gallon of dry sand (4,000 gm.); 25 gm. of $CaCO_3$, 10 gm. of K_2HPO_4, 10 gm. of $Ca(NO_3)_2$, 5 gm. of $MgSO_4$, 3 gm. of KCl and 3 gm. of Fe in the form of a suspension of $Fe(OH)_3$. These chemicals were all free from manganese and pots of sand receiving this treatment served as the controls. Equal numbers of other pots of the sand received 2 gm. each of manganese in the form of $MnCO_3$ and the above-mentioned nutrients. The mineral nutrients and the sand were thoroughly mixed by hand in a large porcelain-lined pan and returned to the respective pots. Equal numbers of plants of soybeans, cowpeas, and sweetcorn were grown in the purified sand, with and without manganese, until they approached maturity. They were then harvested and the dry weights determined and chemical analyses made of the plant material. The results are given in Tables V and VI.

TABLE VI.—*Dry weights of the plants*

Species of plant.	Manganese added.	No manganese added.	Increase due to manganese.
	Gm.	*Gm.*	*Per cent.*
Alaska peas (grown in water cultures)	28. 10	18. 80	49. 5
Soybeans	19. 80	12. 25	61. 6
Cowpeas	26. 50	11. 30	134. 5
Corn	72. 00	59. 00	22. 0

All the plants which grew in the pot cultures containing manganese made a very much better growth than did those from which manganese was withheld. The most striking result was obtained with cowpeas, in

which the increase in dry weight of the plants receiving manganese was 134.5 per cent. All the leguminous plants from which manganese was withheld made a normal growth for the first six or eight weeks; thereafter the young buds and leaves as they unfolded were etiolated and brown specks developed on the etiolated leaves later. After this condition had developed but very little growth was made, and the young and tender parts of the plants died back, while the plants to which manganese was added made a normal growth and no chlorosis developed during their growth. With corn, the only apparent effect produced on the growth of the plants was the failure in the production of dry organic matter. No chlorosis developed on the leaves of the corn, which was harvested after the plants had produced tassels. From these results it appears that leguminous plants are more sensitive towards the lack of manganese than are the nonlegumes.

In the cowpea and corn plants to which no manganese was added, enough of this element was present for a determination. In the other plants to which no manganese was added only a trace of manganese could be detected.

The stalk of the soybean plants which grew in the absence of manganese was the only material in which no manganese could be detected. The plants which grew in the pot cultures to which manganese was added contained a normal amount of this element.

More recent experiments with manganese-free sand cultures at the Kentucky Agricultural Experiment Station have shown the necessity of manganese in both nonleguminous and leguminous plants.

Plate 2, A, shows in a very striking way the beneficial influence of manganese on oats. The plants on the left had no manganese added to the sand in which they grew, while those on the right grew in the presence of manganese.

Plate 2, B, shows Canada field peas. The plants on the left received no manganese in the sand in which they grew, while to those on the right manganese was added, all other conditions being the same.

Sand cultures were carried out with the following species of plants: Wheat, oats, peas, cowpeas, beans, lettuce, tomatoes, onions, spinach, cabbage, carrots, radish, and clover, and similar effects were observed as those described and shown in Plate 2, A, B.

SUMMARY

(1) In the experiments with acid soil which contained approximately one-tenth of 1 per cent of manganese it was found that only small amounts of the total manganese were soluble in water. Applications of more manganese, in the form of the sulphate, to the acid soil caused a decrease in the yields of the crops, whereas like quantities of this compound, when applied to different portions of the same soil, after addition of calcium carbonate, caused an increase in the yields of other plants of the same species.

(2) The occurrence of soluble salts of manganese in an acid soil may be one of the causes of toxicity in such soils as exhibit toxic effects. An excess of manganese sulphate in a soil renders it sterile with respect to the growth of plants. Calcium carbonate in the soil causes a diminution in the toxic effects produced by an excess of manganese sulphate.

(3) In order to demonstrate whether or not manganese is essential for the normal growth of plants, it is necessary that great care should

be taken in the preparation of a manganese-free medium in which to grow plants.

(4) Manganese is intimately associated with compounds of iron, phosphorus, and calcium, and since very small amounts of this element are required for the growth of plants it is quite probable that the contamination of plant nutrients with manganese has hitherto been an unrecognized source of error in determining the necessity of this element in plant economy.

(5) Apparently leguminous plants are more sensitive to the lack of manganese than are the nonlegumes; however, further data obtained by growing nonleguminous plants for more than one generation in a manganese-free medium may be necessary to prove this point.

(6) A very small amount of manganese is required for the normal growth of plants.

(7) Seeds of the plants tested (radish, soybean, cowpea, field pea, and corn) do not contain enough manganese for the growth of the plant to maturity.

(8) The seeds of some plants contain enough manganese to maintain a normal development for the first four or six weeks of their growth; therefore experiments conducted for a shorter time in a manganese-free medium are not likely to give any indications as to the necessity of this element in the growth of plants.

(9) The lack of manganese affects the production of the dry matter in plants, thus indicating that it has some very important function in carbon assimilation.

(10) The etiolated condition of the young and tender leaves and buds obtained in well-controlled experiments indicates that manganese has a function in the photosynthetic process and the formation of chlorophyll.

(11) Manganese apparently is essential for the normal growth and development of plants.

(12) Further investigations on this subject are under way in which an attempt will be made to show the relation of manganese to other important phases of plant and animal life.

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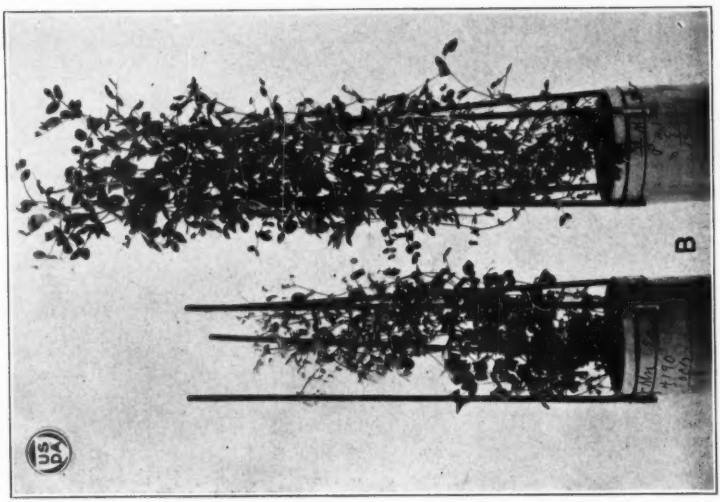
PLATE 1

A.—Radish plants in Volusia silt loam; $MnSO_4$ and plant nutrient added to No. 3, 4, and 5.

B.—Volusia silt loam with $CaCO_3$; $MnSO_4$ and plant nutrients added to No. 4, 5, and 6.

Effect of Manganese on Plant Growth

PLATE I


B

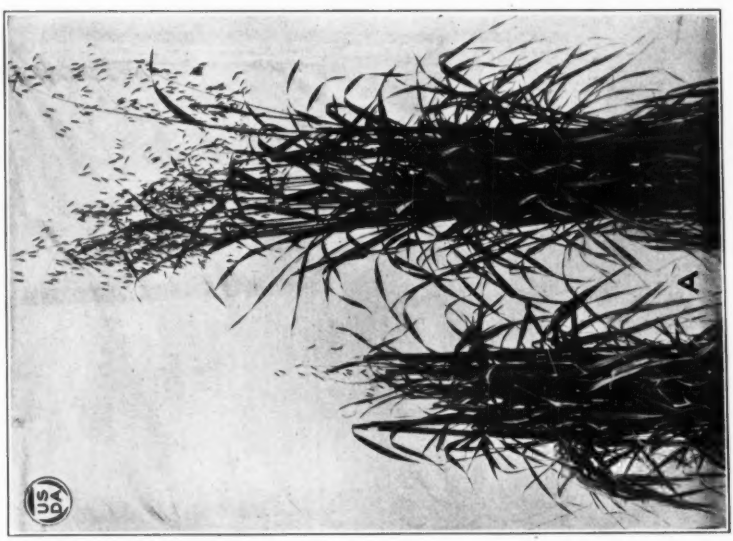

A

PLATE 2

A.—Oats in sand; left, no Mn added; right, Mn added.
B.—Canada field peas in sand; left, no Mn; right, Mn added.

SWEET CLOVER INVESTIGATIONS [1]

By RAY E. NEIDIG, *Chemist*, and ROBERT S. SNYDER, *Associate Chemist, Idaho Agricultural Experiment Station, University of Idaho*

COMPOSITION OF SWEET CLOVER HAY AND SILAGE

Sweet clover has become an important crop in Idaho, not only from the standpoint of feeding value, but also because it is finding a place in a proper rotation system of cropping which is so necessary for diversified farming. Sweet clover is a legume that is comparatively easy to grow in all sections of the Northwest. Its value as a soil improver is rapidly becoming known to progressive farmers both in the humid and semiarid regions. As a pasture crop, sweet clover is finding a much needed place on the farms of the Northwest. Its resistance to drought and its great productiveness under the most adverse conditions warrants a place on every farm. Recent indications are that sweet clover is an excellent crop for alkali districts when the alkali concentration is too heavy for the ordinary hay crops.

Since sweet clover is finding a place in a permanent agricultural system, the following investigation was planned to determine the feeding value at various stages of growth, and also to determine the possibility of utilizing the crop for silage whenever conditions did not warrant making hay. It appeared to the writers that owing to the heavy yields of sweet clover that can be obtained on lands that are not adaptable to the growing of corn or sunflowers, it would prove an excellent substitute for these crops for silage if it could be satisfactorily siloed in Idaho. Consequently the two varieties (*Melilotus alba*) or white, and (*Melilotus officinalis*), or yellow sweet clover, were studied. These two varieties were grown by the Agronomy Department in 1920, and were available for use in these investigations.

STAGES SELECTED

In studying the varieties, two stages were selected for the yellow sweet clover. The first stage was the time when the plant was in blossom. At this stage the plant was 37 inches in height. In all samplings the yield of a definite area was determined, and from this the acre yield was calculated. When the plant was partly in blossom and partly in seed it was cut, and the authors called this the second stage.

The samplings of the white sweet clover were made in three stages: First, before the plant was in blossom and when it was 49 inches in height; second, when the plant was beginning to blossom; and, third, in full blossom and when a few seeds were formed.

The samples were prepared for analysis and for silage by cutting in small pieces in a small silage cutter. A portion of the cut sample was air dried and then analyzed for protein, ether extract, fiber, ash, and nitrogen-free extract. The remaining portion of the sample was used for silage.

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[1] Accepted for publication Nov. 6, 1922.

PREPARATION OF THE SILAGE

Quart milk bottles were used for making the sweet clover silage, since these had been used on other types of silage experiments and found to be very well suited for such work.

The technic of silage making was as follows: A quart milk bottle was packed with the freshly-cut sweet clover and, if lacking in moisture, sufficient water was added to raise the moisture content to 75 or 80 per cent. A large rubber stopper containing a glass tube was placed in the bottle very tightly. This tube was bent at an angle so that its outer end was inserted into a beaker containing mercury. This arrangement allowed the fermentation gases, chiefly carbon dioxid, to escape from the bottle, but did not allow access of air to the silage. At the end of a few weeks, when the major fermentation had been completed, the bottles were opened and the silage examined. The acidity of the silage was determined, also the protein, ether extract, fiber, ash, and nitrogen-free extract.

METHODS USED

In determining the composition of the sweet clovers at various stages of growth, the methods described by the Association of Official Agricultural Chemists were used.[2] The results on the composition are given for the various stages in Table I.

[2] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919. xii, 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at end of chapters.

TABLE I.—*Composition of sweet clover and sweet clover silage*

Variety.	Stage of cutting hay.	Date.	Height.	Acre yield.	Moisture.	Wet basis.					Anhydrous basis.				
						Crude protein.	Ether extract.	Crude fiber.	Ash.	Nitrogen-free extract.	Crude protein.	Ether extract.	Crude fiber.	Ash.	Nitrogen-free extract.
			In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	July 2, 1920	37	11.7	73.5	5.56	0.85	6.8	2.99	10.3	21.09	3.21	25.66	11.28	38.86
	In blossom and partly in seed	July 13, 1920	37	10.9	69.5	5.06	.63	9.99	2.90	11.92	16.59	2.07	32.78	9.49	39.07
	(Silage made from the above two stages of yellow sweet clover):														
	First stage				75.6	4.50	.63	7.48	2.60	9.19	18.44	2.59	30.65	10.61	27.71
	Second stage				72.4	4.38	.49	9.31	2.78	10.64	15.87	1.78	33.71	10.06	38.58
White	Before blossoming	July 2, 1920	49	21	75.4	5.38	.45	7.59	2.65	8.53	21.87	1.83	30.83	10.75	34.72
Original	Beginning to blossom	July 14, 1920	60	14	73.4	5.06	.55	9.01	2.16	9.82	19.02	2.06	33.83	8.12	37.97
Hay	In full blossom	July 22, 1920	60	16.8	69.4	4.25	.57	11.64	2.34	11.80	13.99	1.87	38.02	7.64	38.48
	(Silage made from the above stages of white sweet clover):														
	First stage				77.16	3.75	.51	7.83	2.09	8.66	16.42	2.23	34.20	9.16	37.99
	Second stage				75.32	3.69	.23	8.52	1.99	10.25	14.95	.93	34.50	8.05	41.57
	Third stage				70.64	4.06	.47	10.75	2.16	11.92	15.67	1.61	36.57	7.36	38.79

ACIDITY OF SWEET CLOVER SILAGE

METHODS OF OBTAINING SAMPLES AND DETERMINING ACIDITY

The silage was removed from the containers and 50 gm. were immediately weighed out and dried at 100° C. to determine the moisture content. The remaining silage was placed in a hydraulic press and the juice pressed out. Then 100 gm. of the juice was placed in a vacuum steam distilling flask, together with 5 cm. of normal sulphuric acid. The volatile acids were separated from the nonvolatile acids by distilling in a current of steam in a partial vacuum. Four liters of the distillate were collected, which contained all the volatile acids. The distillate was neutralized with one-tenth normal barium hydroxid and evaporated to a small volume. The volatile acids were then freed from the barium salts by the addition of the theoretical amount of sulphuric acid. After filtering the barium sulphate, the solution was made up to volume and the volatile acids were quantitatively determined, using the methods described by one of the authors in a former publication.

Total acidity was determined by titrating 10 gm. of expressed juice with one-tenth normal barium hydroxide. Nonvolatile acid was determined by subtracting the centimeters of volatile acids found in 100 gm. of silage juice from the total acidity determination. The difference was calculated as lactic, or nonvolatile acid. The results of the volatile and nonvolatile acids are given in Table II, on the silage both with and without moisture.

TABLE II.—*Acidity of sweet clover silage*

Variety.	Stage of cutting.	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. wet silage.			
				Acetic.	Propionic.	Lactic.	Total.
		In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	37	11.7	0.371	0.011	1.399	1.781
Do	In blossom and partly in seed	37	10.9	.694	.054	.853	1.601
White	Before blossoming	49	21	.515	.013	1.353	1.881
Do	Beginning to blossom	60	14	.333	.021	1.250	1.604
Do	In full blossom	60	16.8	.332	.023	1.642	1.997

Variety.	Stage of cutting.	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. dry silage.			
				Acetic.	Propionic.	Lactic.	Total.
		In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	37	11.7	1.520	0.043	5.728	7.291
Do	In blossom and partly in seed	37	10.9	2.516	.020	3.091	5.627
White	Before blossoming	49	21	2.167	.06	5.699	7.926
Do	Beginning to blossom	60	14	1.348	.09	4.063	5.501
Do	In full blossom	60	16.8	1.133	.08	5.599	6.812

DISCUSSION

A comparison between the white and yellow sweet clover shows that the former is the higher yielding variety. It must be borne in mind that both varieties were grown side by side, hence were subjected to the same climatic and soil conditions. The white sweet clover produced a taller and bushier plant than the yellow, which accounts for its greater yield.

In both varieties the first stage contained the larger percentage of water, as was expected. The later cuttings were higher in dry matter. The only significant fact shown (Table I) is that the nitrogen or crude protein decreased and the crude fiber increased, the longer the sweet clover was allowed to grow. This is true for both varieties and clearly demonstrates that if the sweet clover is to be used for hay the early cutting should be chosen rather than the later stage. For silage purposes this conclusion is equally true, for there is slightly less crude fiber and a slightly higher percentage of protein in the earlier stages of cutting.

The acidity determinations show that a normal silage fermentation takes place when sweet clover is siloed. The silage samples all had the characteristic silage acids and odor and were classed as excellent silage.

It is not the purpose of this paper to advise the growing of sweet clover for silage rather than corn or sunflowers, but under certain conditions it is thought that sweet clover can be so used to advantage. In its use as a pasture crop one of the chief difficulties encountered is to pasture sufficient stock upon the sweet clover to keep down the growth. In many cases the sweet clover grows so rapidly that the growth exceeds the pasture requirements and a portion of the sweet clover attains a considerable height. It is thought that in such conditions, where the crop may not make the best hay, it can well be used for silage, since the stalks, if they have been allowed to become too mature, will soften up materially in the silo. Two purposes have been accomplished when a sweet clover crop from pasture land has been used in this manner. First, the food value of the excess growth has been saved and, second, the pasture has been improved by removing the excess growth, and new tender shoots will appear.

The fact that sweet clover is a legume and acts as a soil improver and gives a comparatively high yield of green material under the most adverse conditions of drought, tends to make it a promising crop for the silo in many parts of the Northwest.

GROWTH AND COMPOSITION OF ORANGE TREES IN SAND AND SOIL CULTURES[1]

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The limited control over the factors of nutrition operating in the field (*10*)[2] (*4*) has, perhaps, contributed in a large measure to the heterogeneous array of causes assigned to specific effects. Before any explanation of so-called nutritional disturbances of citrus trees can be accepted, they must be produced experimentally under conditions admitting of scientific analysis. The method subsequently described for growing citrus trees under controlled conditions should make such an analysis possible. The present paper deals with a comparison of the growth and composition of trees when grown in sand and in soil cultures.

It is important to determine, first, whether citrus trees can be grown successfully for several years in sand cultures receiving only inorganic salt solutions; second, whether the growth obtained in sand receiving a nutrient solution (which has been shown to be well suited to the growth of barley) compares favorably with that obtained in soil receiving occasional additions of the same nutrients; and, third, how the composition of the trees grown in sand compares with that of the trees grown in soil.

The importance of the method and the successful results thus far obtained seem to justify a brief description of the way in which these cultures were installed and cared for. The procedure to be described was adopted only after considerable study and experimentation.

As experiments upon trees are usually of long duration, adequate protection and cultural attention are quite essential. A screened enclosure with suitable windbreaks provided the necessary protection against animals and destructive winds. The experiments were conducted in sheet-iron tanks and in large galvanized iron cans. The former were sunk in the ground; the latter were placed in trenches for protection from the direct rays of the sun.

The trenches were 28 inches to 30 inches wide, about 3 feet deep, and were lined on both sides with boards. In the bottom of each trench were two 2-inch by 4-inch pieces of redwood extending the entire length of the trench, which served as supports for the cans and prevented rusting. Thus far it has not been found necessary to provide wooden covers for the trenches. The necessary protection against rain has been obtained by placing frames of roofing paper on wooden trapezes swung by wires from the roof of the enclosure.

Each of the galvanized iron cans (Pl. 1, A) is about 20 inches in diameter and about 26 inches in depth. In order to have a means of weighing the cans, three wrought-iron lugs ¼ inch by 1 inch, each bearing a three-eighths inch hole at the top, were fastened by bolts to the inside of the heavy upper rim. The lugs were equally spaced around the rim and protruded through holes cut in the lid (Pl. 1, B).

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[2] Reference is made by number (italic) to "Literature cited," p. 813-814.

A 4-inch hole was cut in the center of each lid to accommodate the tree. About midway between this center hole and the lid margin, four equally spaced 1¼-inch holes were cut, through which solutions could be poured. These holes were corked when not in use. The inside surface of the can was given a heavy coat of asphalt paint.

The sand used in the cans upon mechanical analysis showed the following percentage composition:

Size by screen (meshes per inch):	Per cent.
Larger than 20 mesh	0
20 to 40	0.33
40 to 60	7.85
60 to 80	54.11
80 to 100	21.63
Smaller than 100	16.08

Chemical analysis of the sand showed 99 per cent silica, 0.2 per cent volatile matter, and 0.8 per cent total water-soluble solids (other than silica or volatile matter), much of which was iron.

A 1-inch pipe, inserted through the lid and extending to the base of the can, provided a means for removing drainage water. About it and to a depth of 4 to 6 inches was placed a well-compacted layer of pure quartz rock crushed to about one-fourth to one-half inch in diameter, to prevent the sand from blocking the drainage system. The sand was added gradually and thoroughly moistened and settled before further additions, care being taken to avoid the use of excessive amounts of water. When the can was filled with sand to within about 2 inches from the top, the excess of water used in filling the can was at once removed through the outlet pipe.

The drainage water was removed by means of powerful air-suction (7) (Gardner-Rix vacuum pump run by one-half horsepower motor, with a capacity of 9 cubic feet per minute), six to eight cans being usually drained in one operation.

A slender iron tube (Pl. 1, B) was inserted through the drainage pipe to the bottom of the can whenever it was necessary to remove drainage water. As a means of excluding sand the lower end of the drainage tube was covered with a piece of fine tin screen. Excellent results over a period of years are readily obtained if the drainage water is removed as rapidly as it enters the drainage pipe. Plate 1, B, shows one of the drainage bottles removed from its wooden rack and raised so as to show the general scheme used in removing the drainage water.

The air outlet from each drainage bottle is provided with a gas cock by means of which the rate of flow into each bottle can be regulated.

A 2-year-old Valencia orange tree was planted in each can after the soil was thoroughly removed from the roots by repeated washing. The root system was pruned until only a small part remained. The upper portion of the tree was also pruned (Pl. 1, A); the lateral shoots and the leaves were removed and the cut surfaces covered with asphalt paint. Where it was not desired to exclude calcium from the culture, the trees were given a coat of whitewash. Where whitewash was not applied, a cylinder of wire netting was placed about the tree and the east and south sides were protected with cheesecloth attached to the wire. When the trunk was shaded by new shoots, the cloth protector was removed, preferably during cloudy or foggy weather. After the tree was planted in the sand and the lid was placed on the can a piece of white oilcloth, with a hole in the center, was slipped down over the tree trunk and was fastened to the lid with asphalt paint. A thin wad of cotton was placed

between the oilcloth and the tree. A cylinder of heavy brown wrapping paper was placed about the base of each trunk to give added protection from sunburn.

Where soil was employed the cans were prepared as stated before, which made possible the removal of salts while the tree was in the soil. Wilting-point determinations were made on the soils and they were then brought to a suitable water content prior to being gently compacted in the cans.

In the case of sand, additions of carbon-treated distilled water were continued until the first indications of drainage water appeared in the drainage pipe. Air suction was then applied. Nutrient solutions (6 to 9 liters or more) made up with carbon-treated distilled water were then added and the drainage continued until complete. If drainage water appeared later in the bottom of the cans, the air-suction was again applied until all the free solution had been removed. The problem of drainage was subsequently simplified by providing a means for gravity drainage. The cans were placed on supports over the trenches and a short iron pipe was inserted into the bottom of each can. As drainage water percolated to the bottom of the can, it dripped through the short pipe into a bottle standing in the trench. This arrangement is decidedly advantageous because it precludes the accumulation of water in the bottom of the can and also materially lessens the amount of labor required in caring for the cultures. The cans were shielded from the direct rays of the sun by housing them in a wooden box. The treatments were applied every one to three weeks as conditions required. During hot weather a small tree growing in sand (about 225 pounds of moist sand) may require from 24 to 30 liters of distilled water each 20 days. A Barnstead still capable of giving 5 gallons of distilled water per hour, with a block-tin-coated tank capable of storing 2,000–2,500 liters of distilled water, and 24 large carboys for treating the distilled water with carbon black together with filtering facilities, have made it possible to maintain in operation 136 trees in cans as well as several trees in tanks of sand.

In the case of cans of soil when drainage water may not be desired, the weighing device patterned after that of Briggs and Shantz (*2*) is used (Pl. 1, C). A windlass with wire rope and pulleys is mounted on a frame work of 2-inch galvanized-iron piping to which wide swivel castors are attached. Two spring scales with circular disks, 500 and 600 pounds capacity, respectively, are used for bringing the cans to constant weight. As the trees develop in cans of soil, it is necessary to make allowance for the increased weight of the tree in order to maintain suitable moisture content of the soil. The criteria used for this purpose are, appearance of the tree, presence or absence of drainage water, and appearance of the surface of the soil upon slightly raising the lid, or preferably by examining a sample of the soil. It is a good practice occasionally to raise the lids of the cans of soil to ascertain whether channels have been formed in the surface layers. It is best to weigh the cans frequently, since if large additions of solution are required, channels may appear throughout the soil and drainage water may appear without much of the soil being appreciably above the wilting point.

In order to carry on experiments with trees for a longer period of years, containers of a larger capacity than that of the cans are necessary. Tanks of two sizes are being used. The smaller are 3 feet 8 inches in diameter by 4 feet deep (Pl. 2, A), the larger are 7 feet 8 inches in diam-

eter by 4 feet 4 inches deep (Pl. 3, A). The bottom of each tank tapers toward the center, which is 6 inches deeper than the circumference.

The center of the bottom of each tank bears a perforated brass plate, beneath which is attached a 4-inch elbow that connects with 2-inch galvanized-iron piping leading to a trench. Each tank has its individual drainage outlet. Crushed quartz rock was placed in the bottom of each tank above which is placed sand or soil, as the case may be.

The nutrient solution employed in the present experiments was that used by Hoagland (5) in the culture of barley. Filtered carbon-treated distilled water was used whenever water was required. The stock solutions were made up as follows:

	Gm.
Solution A.	
KNO_3	1,200
$MgSO_4+7H_2O$	1,800
NaCl	55
H_2O to give volume of 18 liters.	
Solution B.	
$Ca(NO_3)_2+4H_2O$	2,600
H_2O to give volume of 18 liters.	
Solution C.	
KH_2PO_4	900
H_2O to give volume of 18 liters.	
Solution G.	
$MnSO_4+4\ H_2O$	0.406
H_2O to give volume of 2 liters.	

The nutrient solution was prepared by using the following amounts of the stock solutions: 55 cc. of solution A, 65 cc. of solution B, 30 cc. of solution C, and 20 cc. of solution G, made up to 10 liters with distilled water.

The nutrient solution as thus made had a P_H of 5.2 and an osmotic pressure of 0.728 atmospheres. The composition of the nutrient solution was as follows:

Parts per million.

NO_3	SO_4	K	Ca	PO_4	Mg	Cl	Na	Fe	Mn	Total
718	216	185	159	105	54	10	7	1	0.1	1455

The iron was not added to the nutrient solutions until they were ready to be added to the cultures. As a rule 5 to 10 parts per million of Fe were used in the nutrient solution, the plan being to have sufficient Fe present, as indicated largely by the appearance of the tree. Small quantities of ferric tartrate were subsequently added whenever the cultures were irrigated.

The trees were planted on May 21, 1920, and were removed September 20, 1921. Trees 1 and 2 were typical of the sand cultures (1–5), and tree 85 was typical of the soil cultures (84–88). The tree in can 2 was much smaller than the others in its series at the start, although it made excellent growth.

Plate 2 B, is a photograph of trees 1, 2 and 3 taken August 20, 1921. The photograph indicates that citrus trees can be grown in pure sand to which no organic matter has been added, except the small amount as iron tartrate. Plate 4 shows the leaves of tree No. 3 which were typical of the series, being dark green in color and giving no indications of malnutrition.

This is of interest in connection with the results (1) of field studies in which it was found that low humus content of the soil was associated with the mottling of orange trees and also that applications of organic

fertilizers tended to reduce the amount of mottling. Although the authors have grown 23 orange trees in sand cultures and 5 in soil receiving various concentrations of an inorganic nutrient solution, no typical mottle-leaf has been found as yet on any of the trees.

A word of caution is here necessary as regards the choice of orange trees for sand cultures. It seems best to use trees which are free from mottle-leaf, although trees which previously bore mottled leaves may present no difficulties after their behavior is once known. When mottled trees have been used in sand cultures receiving Hoaglands solution, and the leaves, shoots, rootlets, etc., removed as before stated, the first cycle of growth may bear a few mottled leaves most of which may recover, but the subsequent cycles have thus far been free from any evidence of mottling.

In order to observe the effect of the sand on the P_H of the solution, some of the nutrient solution was placed in liter Erlenmeyer flasks closed with rubber stoppers. Sand was added to one flask, shaken, and allowed to stand from December 29, 1920, to January 24, 1921. The P_H of the nutrient without sand had not changed, while that in contact with sand had increased from 5.2 to 5.9. Other culture solutions treated in this manner gave similar results. Shive (*9*) has reported that the reaction of his nutrient solution was not markedly altered by contact with unwashed sand. He finds that sand has an initial adsorptive effect but this is soon satisfied in sand cultures where the nutrient solution is renewed frequently. The increase in the P_H of the solution in contact with the sand was probably due to chemical reactions and not to adsorption.

The adsorptive property of unwashed sand probably was due to the very finely divided colloidal or semicolloidal material which was removed from the sand in the process of washing. The P_H values of percolates from the cans were determined from time to time to learn whether material changes ensued after prolonged contact with the sand and tree roots. The results of several determinations on percolates from cans 1 and 2 are given in Table I. The first two determinations showed the increase when a nutrient solution having an initial P_H of 5.2 was allowed to percolate through the sand of one of the cans. The other determinations show the changes in P_H when distilled water was allowed to percolate through the sand. These determinations are more variable, owing in part to the fact that the distilled water washed out varying quantities of the residual nutrient solution. In every case the P_H of the percolate tends to move in the direction of neutrality.

TABLE I.—*P_H values of nutrient solution and percolate from trees grown in sand cultures*

Date.	Application.	Amount of percolate collected.	Tree No.	P_H of percolate.
1920:		*Cc.*		
Nov. 2	Nutrient solution	100	1	6.8
Do	do	100	2	6.7
Nov. 29	Distilled water	500	1	7.25
Do	do	500	2	6.6
1921:				
Mar. 28	do	500	1	5.4
Do	do	500	2	6.5
Apr. 20	do	10	1	5.9
Do	do	10	2	6.7

The osmotic pressure of the sap of leaves from trees in the sand cultures was 20.80 atmospheres and that of leaves from the soil cultures was 21.03 atmospheres. The P_H of the former was 6.00 and that of the latter 5.80. We can not regard these differences as very significant.

Can 85, as well as the other cans of the series 84–88, inclusive, contained soil obtained from an uncultivated hillside near Riverside, Calif.

The hygroscopic moisture content of the soil was determined and the soil was brought to a suitable moisture content.

The cans of soil were brought to a given weight at frequent intervals by the addition of distilled water or nutrient. During the experimental period (May 21, 1920, to September 20, 1921) can 85 received the following additions of salts.

Salts.	Amounts added.
	Gm.
KNO_3	9.89
$MgSO_4+7H_2O$	14.85
NaCl	0.46
$Ca(NO_3)_2+4H_2O$	25.27
KH_2PO_4	4.05
$Fe_2(C_4H_4O_6)_3$	0.17
$MnSO_4+4H_2O$	0.0108

Plate 3, B, shows a typical tree in the soil series (84–88). The growth in sand compared very favorably with that in soil.

In November, 1920, after the addition of distilled water to cans 1 and 2, the first 500 cc. of percolate obtained from both cans were analyzed for the PO_4 and NO_3 content. The results given below indicate that an excess of both ions was present.

Parts per million.

	PO_4	NO_3
Can 1	55.7	1,329
Can 2	65.5	886

The soil in can 85 received only distilled water from May, 1920, to May, 1921, after which tap water was used. Hoagland's nutrient was occasionally added. Soil samples were taken from can 85 on October 8, 1920, and also in September, 1921. A partial analysis of a 1 to 5 aqueous extract of the dried soil gave the following results when expressed in terms of the dried soil:

Soil samples from can 85, October 8, 1920.

	Parts per million.		Parts per million.
Ca	22	Cl	18
Mg	9	HCO_3	76
Na+K (calculated as Na)	3	SO_4	15
Total solids as sulfates	128	NO_3	40
SiO_2	19	P_H of extract (colorimetric)	6.6

Soil samples from can 85, September, 1921.

	Parts per million.		Parts per million.
Ca	18	Cl	31
Mg	8	HCO_3	61
Na	4	SO_4	44
K	27	NO_3	22
Total solids	313	P_H of extract (colorimetric)	6.6
SiO_2	36		

The trees were removed from cans 1, 2, and 85 on September 20, 1921. Tree 85 was free from mottle-leaf, as were all the trees in the soil series, and was making excellent growth. Plate 5, A, shows the air-dried root systems of trees 1 and 85, grown in sand and in soil respectively. It is evident that starting with trees of nearly similar size, as good growth may be obtained in sand as in soil cultures. The root systems obtained in the sand cultures were somewhat coarser than those obtained in the soil cultures, the root system in the soil cultures being extremely fibrous (Pl. 5, B).

The initial operation in removing a tree from the can was to remove and count the leaves, which were then cleaned with a dry cloth. The shoots were removed at the trunk and after being cut up finely were placed in paper bags. The lid of the can was then removed and soil samples taken. The root system was obtained intact by washing the sand or soil from the tilted can with a strong jet of water. After carefully removing the adhering sand or soil from the rootlets with tap water, they were washed with distilled water. The upper portion of the tree axis was sawed off close to the first lateral root and was designated as the trunk. In cases where the trunk had been previously coated with whitewash it was cleaned with a soft scrubbing brush and tap water and then rinsed with distilled water.

When air dry the trunk was further cleaned with a soft wire brush. All new root laterals formed subsequent to the planting of the tree were removed, placed in bags, and were designated as "rootlets." The root axis, together with any pruned laterals which were present when the tree was first planted in the can, were designated as "root." The root was thoroughly cleaned with the use of a soft wire brush.

The several portions of each tree were dried to constant weight at 60° to 70° C. The rootlets were then shaken in the upper compartment of a set of soil sieves, to remove as much adhering matter as possible. In spite of the care taken in cleaning the rootlets the amount of silica still adhering to them was sufficient to require the calculation of analytical results to a silica-free basis. In the case of trees grown in soil the task of obtaining clean rootlets was so difficult that determinations of ash constituents were omitted from the analyses.

The dry weight, number of leaves, and water transpired for each of the three trees are given in Table II.

TABLE II.—*The dry weight of various portions of the trees, the number of leaves, and the water transpired*

Tree No.	Number of leaves on tree.	Dry weight (60° to 70° C.).						Total nutrient added.	Total distilled water added.	Total drainage water.	Transpiration.	Total transpiration per total dry weight of trees.
		Leaves.	Shoots.	Trunk.	Root.	Rootlets.	Total.					
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Liters.*	*Liters.*	*Liters.*	*Liters.*	
1	996	237.1	122.5	194	212	136	901.6	243	125	139.5	228	0.253
2	302	76.5	20.7	126	64	57	344.2	187	83	173	97	0.282
85	1,042	205.9	96	140.5	135	129.5	706.9	[a] 27	[b] 29	0	166	0.235

[a] After May, 1921. [b] May, 1920, to May, 1921. Tap water after May, 1921, 110.2 liters.

When the trees were removed from the cans, their appearance was noted. Tree 1 was an excellent tree with large top and splendid foliage. A few leaves tended to split along the midribs. The root system was large, well developed, and filled practically the entire can.

Tree 2 was the smallest in its series, but was smallest when planted. The leaves were excellent. The lowermost portion of the root axis had died, but a large mass of healthy laterals developed directly above the dead portion. Tree 85 was splendidly developed and was typical of its series. The rootlets were much more finely divided than in the sand series (Pl. 5, A, B).

The dry leaves, shoots, and rootlets were passed through a large hand mill, and any pieces remaining underground were removed. The trunk and root portions were sawed into thin sections, the sawdust being used to represent the trunk and root, respectively.

When ready for analysis the samples were thoroughly mixed and so far as the material available permitted duplicate determinations were run. The methods of analysis employed were essentially those used by Kelley and Cummins (*6*) in their analyses of citrus material. Manganese was determined colorimetrically by the persulphate method, which is very satisfactory for small amounts (*11*). Iron was also determined colorimetrically. A few sodium and potassium determinations were made, using the filtrate after the Fe, Al, PO_4, Ca and Mg were removed, but the analyses indicated that low results were obtained. Subsequently more satisfactory results were obtained by using the filtrate from the sulphate determination.

The analytical results for the several portions of each tree are presented in Tables III to VII, inclusive. The data have been calculated as percentage of dry matter and as percentage of ash. The figures given are the averages of two closely agreeing duplicates. It is seen that the total percentage of the ash approximates 100 in most cases, but in none reaches it. Those who are familiar with the details of analytical work of this kind appreciate its difficulties. If the reader be one who insists upon total percentages equaling 100 the authors can merely say, in common with other workers in this field, "peccavimus."

TABLE III.—*Analyses of leaves*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	3.24	3.14	2.15	.....	.....	.....
S	0.31	0.30	0.29	.....	.....	.....
P	0.19	0.20	0.15	.....	.....	.....
Ash	14.37	14.32	13.72	.....	.....	.....
K	3.54	3.56	1.44	24.59	24.91	10.51
Na	0.13	0.21	0.16	0.90	1.43	1.16
Ca	2.71	2.66	3.94	18.85	18.57	28.66
Mg	0.31	0.26	0.37	2.14	1.75	2.65
Mn	0.004	0.004	0.002	0.03	0.03	0.02
Fe	0.025	0.04	0.03	0.17	0.26	0.23
Al	0.17	0.14	0.08	1.14	0.97	0.59
Cl	0.04	0.05	0.02	0.26	0.31	0.15
CO_3	.....	.....	.....	40.70	40.93	42.64
SO_4	.....	.....	.....	3.18	3.17	3.91
SiO_3	0.39	0.43	0.47	2.75	3.02	3.43
PO_4	.....	.....	.....	3.92	4.02	3.23
Total	.....	.....	.....	98.63	99.37	97.18

TABLE IV.—*Analyses of shoots*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	1. 55	1. 76	0. 79	.....	.....	.....
S	.....	.....	.....	.....	.....	.....
P	0. 21	0. 23	0. 14	.....	.....	.....
Ash	6. 81	8. 30	5. 69	.....	.....	.....
K	1. 15	1. 22	0. 55	16. 85	14. 47	9. 79
Na	0. 13	0. 27	0. 08	1. 88	3. 23	1. 47
Ca	1. 46	1. 88	1. 58	21. 34	22. 62	27. 71
Mg	0. 21	0. 22	0. 18	3. 06	2. 67	3. 19
Mn	0. 001	0. 002	Trace.	0. 02	0. 02	Trace.
Fe	0. 02	0. 02	0. 05	0. 32	0. 23	0. 89
Al	0. 10	0. 10	0. 07	1. 46	1. 15	1. 15
Cl	0. 01	0. 01	0. 01	0. 14	0. 11	0. 21
CO_3	.....	.....	.....	35. 90	39. 91	40. 95
SO_4	.....	.....	.....	3. 13	2. 38	3. 01
SiO_3	0. 11	0. 12	0. 16	1. 53	1. 48	2. 75
PO_4	.....	.....	.....	8. 58	7. 64	6. 45
Total	.....	.....	.....	94. 21	95. 91	97. 57

TABLE V.—*Analyses of trunks*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	0. 05	0. 15	0. 40	.....	.....	.....
S	0. 03	0. 07	0. 03	.....	.....	.....
P	0. 09	0. 11	0. 07	.....	.....	.....
Ash	2. 85	3. 22	2. 08	.....	.....	.....
K	0. 39	0. 31	0. 22	13. 57	9. 40	10. 57
Na	0. 18	0. 24	0. 11	6. 03	7. 45	4. 86
Ca	0. 68	0. 80	0. 51	23. 91	24. 77	24. 20
Mg	0. 09	0. 09	0. 07	3. 12	2. 77	3. 09
Mn	Trace.	Trace.	Trace.	.....	.....	.....
Fe	0. 003	0. 02	0. 01	0. 11	0. 40	0. 51
Al	0. 09	0. 16	0. 09	3. 06	4. 92	4. 49
Cl	Trace.	0. 001	0. 006	.....	0. 04	0. 28
CO_3	.....	.....	.....	38. 02	34. 18	33. 45
SO_4	.....	.....	.....	1. 96	3. 46	4. 51
SiO_3	0. 04	0. 11	0. 06	1. 31	3. 15	2. 94
PO_4	.....	.....	.....	5. 11	3. 70	3. 97
Total	.....	.....	.....	96. 20	94. 24	92. 87

TABLE VI.—*Analyses of roots*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	1.01	0.98	0.82	.....	.....	.....
S	0.01	0.13	0.06	.....	.....	.....
Ash	3.03	3.08	2.77	.....	.....	.....
K	0.36	0.33	0.28	11.74	10.55	10.05
Na	0.14	0.10	0.07	4.33	3.15	2.42
Ca	0.72	0.71	0.70	23.77	23.06	25.26
Mg	0.08	0.09	0.05	2.69	2.87	1.74
Mn	Trace.	Trace.	Trace.	.....	.....	.....
Fe	0.02	0.02	0.02	0.50	0.49	0.63
Al	0.08	0.13	0.07	2.41	4.10	2.35
Cl	0.003	0.006	0.016	0.09	0.17	0.59
CO_3	.....	.....	.....	38.28	33.15	41.17
SO_4	.....	.....	.....	2.51	4.47	2.38
SiO_3	0.08	0.14	0.15	2.75	4.68	5.35
PO_4	.....	.....	.....	7.74	9.43	5.55
Total	.....	.....	.....	96.81	96.12	97.49

TABLE VII.—*Analyses of rootlets calculated to a silica free basis*

Element.	Expressed as dry matter.		Expressed as ash.	
	Tree 1.	Tree 2.	Tree 1.	Tree 2.
N	2.26	2.22	.....	.....
S	0.29	0.27	.....	.....
P	0.69	0.56	.....	.....
Ash	11.93	11.19	.....	.....
K	2.04	2.38	17.14	21.24
Na	0.12	0.09	1.02	0.82
Ca	2.36	2.01	19.78	17.98
Mg	0.36	0.34	3.06	2.98
Mn	0.02	0.02	0.16	0.19
Fe	0.04	0.04	0.30	0.28
Al	0.36	0.36	3.03	3.22
Cl	0.12	0.13	0.89	1.12
CO_3	.....	.....	22.25	24.00
SO_4	.....	.....	8.89	8.46
PO_4	.....	.....	19.65	14.62
Total	.....	.....	96.17	94.91

In the analyses reported by Kelley and Cummins (*6*) the carbonates of the ash were not determined. At their suggestion these determinations have been included in the present studies. With rootlets it is practically impossible to remove all of the adhering sand, and consequently the results obtained for the rootlets (Table VII) have been calculated as percentages on a silica-free basis. The rootlets of tree 85 (soil culture) could not be sufficiently freed from adhering soil to permit of satisfactory analyses.

The precautions necessary in distinguishing between cause and effect, when interpreting analytical data of plant tissue, have already been emphasized (*6*). In the dry matter there is a progressive decrease in the percentage of total nitrogen in the sand and soil cultures as we pass from the leaves toward the root, where there may be a slight increase which becomes augmented in the rootlets, whose percentage is considerably below that found in the leaves but greater than that found in the shoots.

The percentage of total sulphur was approximately the same for the corresponding parts of the trees in both sand and soil, the shoots and root in every case containing but a trace.

The percentage of total phosphorus was usually somewhat greater for the sand than for the soil cultures. The leaves and shoots contained approximately the same percentage of total phosphorus; while the trunk and roots contained considerably less, with an appreciable increase in the rootlets above that of the roots.

We find the total ash content of the parts examined to be greater in each case for the sand than for the soil culture. The ash of the leaves was approximately double that of the shoots which in turn was more than double that of the trunk and root.

No significant difference as regards total ash existed between the trunk and root. It is of interest to note that the leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen, which no doubt indicates that here the assimilatory processes are most active.

The percentage of CO_3 constitutes approximately four-tenths of the total ash, except in the rootlets, where it is only about two-tenths. The large percentage of CO_3 found in the ash of various parts of the citrus tree may indicate that not only the leaves, but the other portions of the tree as well, contain large quantities of organic acids.

The percentage of SiO_2 in the ash varies widely in the various parts.

The percentage content of potassium (K) in the ash of citrus leaves obtained in the field (Kelley and Cummins (*6*)) was approximately 20 per cent when 1 week old, 13 per cent when 6 weeks old, 6 per cent when 6 months to 2 years old, and 2 per cent when 3 or more years old. In the sand cultures the potassium constituted approximately 25 per cent of the total ash of the leaves, about 17 per cent of that of the shoots and about 11 per cent of that of the trunk and roots, respectively. In the soil culture, the potassium in the ash of all parts of the tree examined was approximately 10 per cent. The leaves collected from trees in these cultures probably ranged from 3 to 17 months of age and therefore represent a mixed sample so far as age is concerned. The data for potassium indicate that not only the age of the leaf but also the composition of the medium in which the tree grew may influence the percentage content of potassium in the various parts. The data do not necessarily conflict therefore with those obtained by Kelley and Cummins.

The sodium (Na) content of the ash of the leaves and shoots is relatively small compared with that of potassium. The difficulty of accurately determining a small amount of Na in presence of considerable amounts of K will be appreciated by those familiar with analytical work. The data show that the trunk and root contain a much larger percentage of sodium in the ash than either the leaves or shoots. As the location of the sodium storage in citrus trees is of considerable interest and the data thus far obtained are too meager to justify any far-reaching assumptions,

we will reserve further discussion of the matter until the results of experiments in sand, in which the culture solution contained large amounts of sodium chlorid, have been reported.

The percentages of calcium (Ca) in the ash of the leaves and shoots of the soil culture were higher than for the sand cultures; the trunk and roots contained, approximately, the same percentages respectively in all three cultures. The calcium in the total ash varied from 18 to 30 per cent in the various parts in both sand and soil cultures. The percentage content of calcium in the leaves of the sand cultures was considerably lower than that of the soil culture, while the potassium relation was the reverse of this. These as well as previous analyses have shown that citrus trees have a large capacity for absorbing calcium. Coville with the aid of Breazeale (*3*) has reported the percentage of calcium oxid in the ash of other trees as compared with that of citrus. They have found that the percentage of CaO in dried freshly fallen leaves ranged from 1.73 per cent in Red Oak (*Quercus rubra*), to 6.77 per cent in orange (*Citrus aurantium*) with 10 other species of forest trees having intermediate values, basswood (*Tilia Americana*) attaining the second highest percentage, or 4.50 per cent.

It is of interest to note the consistently low percentage of magnesium (Mg) in the ash of the various parts of the trees in the sand and soil cultures.

The percentage of aluminum (Al) in the ash of the trunk, root, and rootlets is greater than that of the leaves or shoots. The small percentage of aluminum in the ash of the leaves and shoots indicates that these parts of the trees were thoroughly cleaned from adhering dust. As the nutrient medium used in the sand cultures contained no aluminum, we would expect the trees in these cultures to contain but little.

The percentage of iron (Fe) in the ash of the various parts of the three trees ranged from 0.2 to 0.9 per cent. The percentage of manganese (Mn) was usually very low. The percentage of chlorin (Cl) in the ash ranged usually below 1.2 per cent. The percentage of sulphate (SO_4) present in the ash was considerably higher than that of chlorin, being 2 to 5 per cent, although the rootlets in the sand culture showed over 8 per cent.

The percentage of phosphate (PO_4) in the ash of the shoots was considerably higher in both the sand and soil cultures than that of the leaves, with a decrease in the trunks and an increase in the roots. The ash of the rootlets in the sand cultures contained about 17 per cent phosphate as compared with 8 per cent in the ash of the shoots and 4 per cent in that of the leaves.

It is of interest to note the high percentage content of K, Ca, and PO_4 in the ash of the rootlets in the sand culture, constituting over 50 per cent of the ash of the rootlets, with a marked reduction in the percentage of CO_3. The percentage of silica-free ash in the rootlets approaches that in the leaves and is much greater than that in the shoots, trunk, or root.

The percentage of PO_4 in the ash of rootlets was greater than for any other portion of the tree. The largest percentages of mineral constituents appear to be deposited in those parts of the tree in which the assimilatory processes are most active.

The sap of citrus leaves possesses the power to neutralize considerable base. Kelley and Cummins (*6*) have shown that 10 cc. of normal leaf sap required 3 cc. of N/10 alkali for neutralization, while mottle-leaf

sap required 7.05 cc. of N/10 alkali. Protein formation or decomposition may bring about the formation of organic acids (*8, p. 237–249*). Since a large portion of the calcium in many plants is used for the neutralization of acids, there should be more calcium in plants producing large amounts of acid. The analyses indicate that this may be the case with orange trees, namely, that one of the chief functions of calcium in the tree may be the neutralization of the organic acids. Opportunity to test out this hypothesis further will be afforded in cultures now growing.

SUMMARY

(1) Data have been presented to show that the nutrition of young trees may be studied in a quantitative way under controlled conditions by the procedure described.

(2) The reaction of the nutrient solution used shifted from P_H 5.2 to 5.9 when the solution was in contact with sand, and further toward the neutral point when in contact with sand containing citrus rootlets.

(3) The relative amounts of new growth produced by the trees grown in sand or soil cultures were quite similar when one estimates the ratio between total dry weight and the dry weight of corresponding portions. The ratio of total transpiration to total dry weight of tree was approximately the same in both cases. The rootlets of the trees grown in soil were much more finely divided than those grown in sand.

(4) Analyses of the trees showed both a high calcium (Ca) content and a high Ca/N ratio.

(5) The percentages of total phosphorus in leaves and shoots were approximately equal.

(6) The leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen.

(7) The ash obtained from all parts of the tree contained large amounts of carbonate, although the rootlets contained less than the other portions.

(8) The ash of all parts of the trees was markedly rich in potassium and relatively poor in sodium. The former was relatively uniform in the various parts of the tree, but the distribution of the latter was more variable.

(9) The various parts of the trees likewise contained large amounts of calcium and relatively small amounts of magnesium. Both constituents show fairly uniform distribution throughout the tree.

(10) The distribution of sulphate in the ash of the various parts of the tree is quite uniform with the exception that the rootlets contained nearly three times as much as the other portions of the tree.

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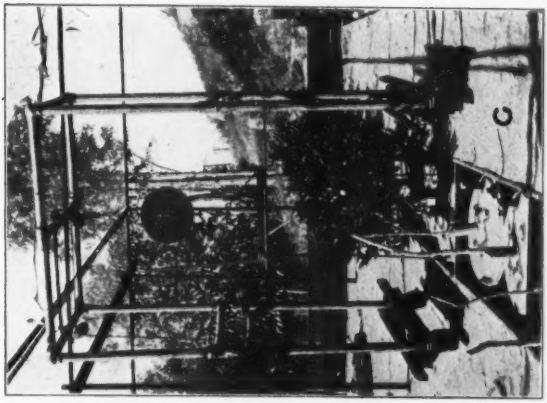
PLATE 1

A.—A typical trench with cans of sand containing orange trees just beginning growth.

B.—Showing attachment of air-suction hose to one of a series of drainage bottles. The drainage tube is removed from the drainage pipe. Can and bottle are raised considerably for photographic purposes.

C.—Device for weighing cans.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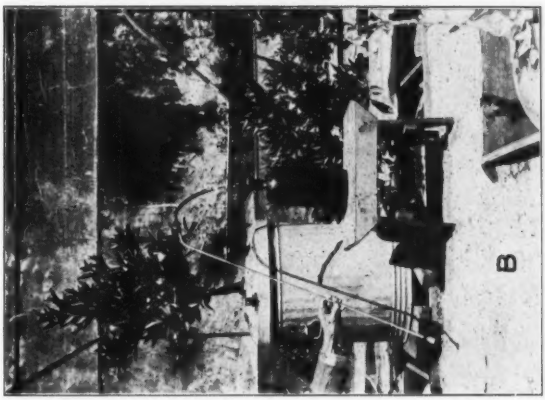
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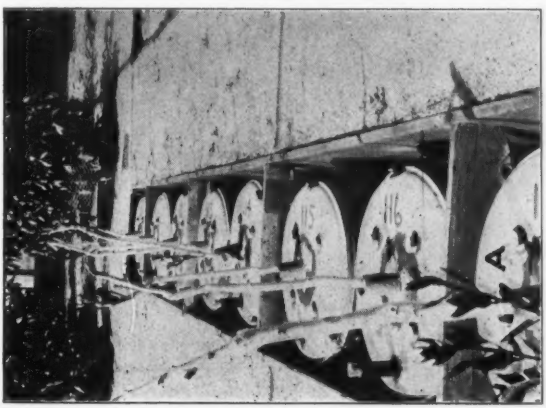
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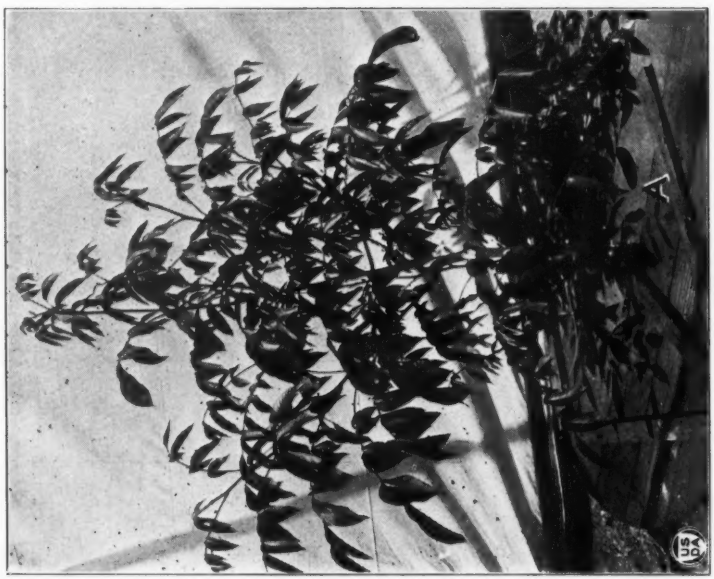
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PLATE 2

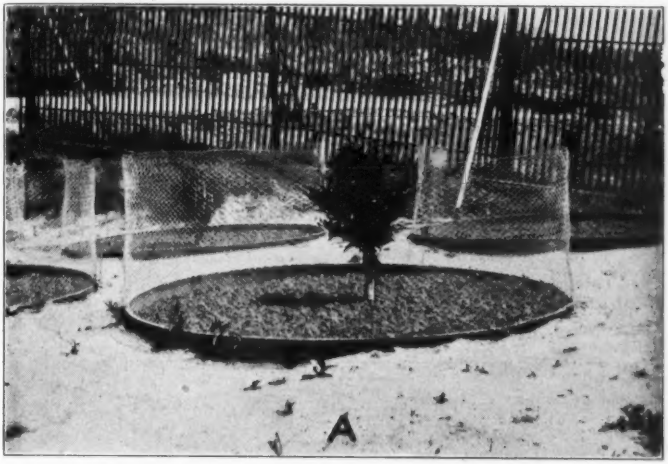
A.—Walnut tree after six months in covered tank of sand that receives a nutrient solution made up with distilled water.

B.—Valencia orange trees after 15 months in cans of sand to which Hoagland's nutrient has been added.

PLATE 3

A.—Valencia orange tree in large tank of soil. Seven feet 8 inches in diameter and 4 feet 4 inches in depth.

B.—Growth typical of trees in soil series 84–88 receiving Hoagland's nutrient at intervals.


A

B
USDA

1
2
3
4
5
6
7

PLATE 4

Orange leaves typical of the sand series. The lighter areas are due to reflection of the artificial light. Scale of inches at bottom of photograph.

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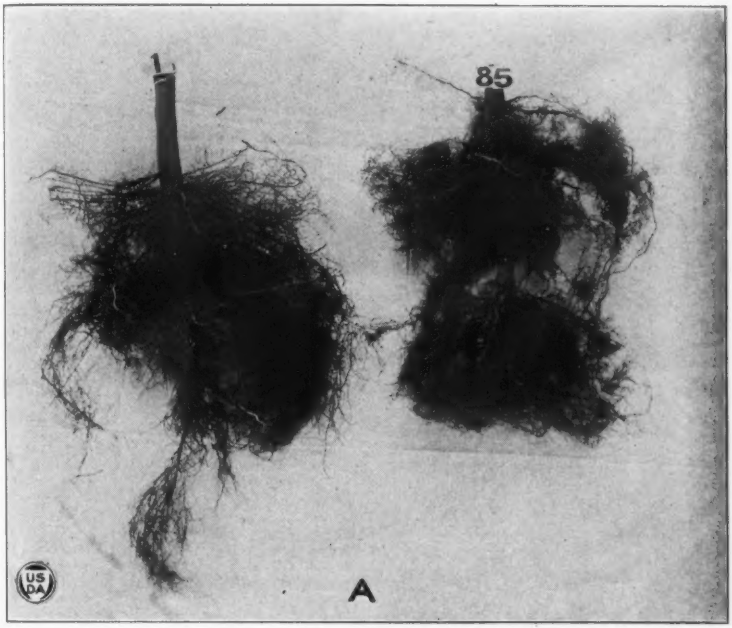
PLATE 5

A.—Air-dried root systems of trees 1 and 85, in sand and soil, respectively, receiving Hoagland's nutrient solution.

B.—Root systems of trees 3 and 86, in sand and soil, respectively, receiving Hoagland's nutrient solution. Note the more finely divided root systems in the soil culture.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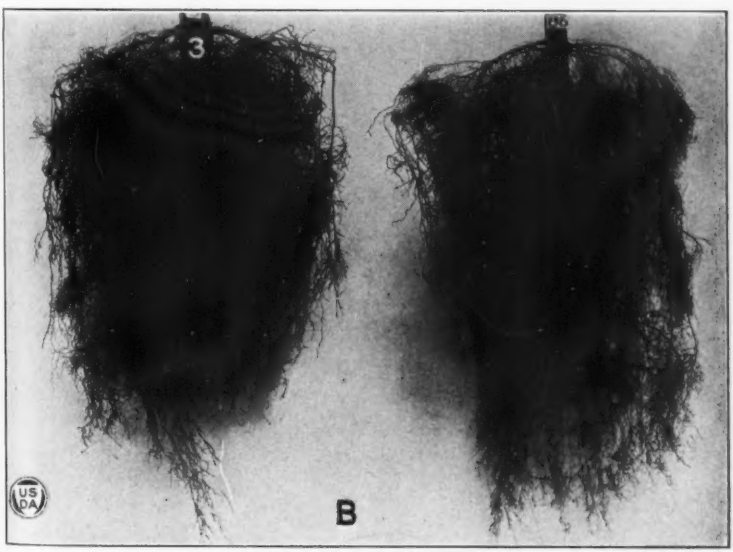
85
A


3
B